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THE FUNCTION OF THE LIPIDS IN MILLING AND BAKING[1]

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Most of the research on wheat and flour has been centered on the proteins in as much as the quality of gluten (the characteristic protein of wheat) has seemed in large measure to determine the desirability of any given flour for bread-making purposes. Until recently little work had been done on the lipids and their influence in milling and baking. Our knowledge on this subject is still woefully incomplete.

Milling itself is an entirely mechanical procedure and the lipids do not affect the actual mechanical operations of breaking, sifting, and air purification except insofar as they alter the physical characteristics such as friability and specific gravity of the various parts of the wheat kernel.

The ideal in milling is the complete removal of both the bran and the embryo from the endosperm of the wheat because, by themselves, neither bran, the outer envelope of the kernel, nor germ is capable of making a loaf of bread when mixed with water, yeast, and salt and handled in the usual manner. Neither bran nor germ can retain the carbon dioxide gas developed in fermentation to produce the well-risen, palatable bread which can be made with white flour.

Another reason why the miller attempts as complete a removal as possible of the bran and germ is that these parts of the berry decrease the keeping quality of flour, owing, in part, to the proportionally higher fat content of the bran and germ as compared to the endosperm.

The higher lipid content of the germ, in particular, makes its physical character different enough to allow an easy, though incomplete, separation from the flour.

The distribution of the lipids in the various separations made on a modern mill from hard spring wheat is as follows.

[1] Paper presented at the Cincinnati meeting of the American Chemical Society, April 8–12, 1940.

	Percent Lipids (Alcohol-ether extract) (Calculated to 13.5% moisture)
Wheat	3.0
Short patent	1.3
Straight	1.6
Clear	2.7
Low grade	3.2
Bran	6.5
Shorts	7.5
Germ	15.5

These figures are only approximate and will, of course, vary with the type of wheat, the percentage extraction, and the method of milling.

The better grades of flour, from both a storage and a baking standpoint, have the least possible amount of bran and germ and, therefore, a rather low lipid content. Even though low, the lipids of flour are of considerable importance.

Lipids of Wheat Products

Wheat germ makes up about 2% of the weight of the wheat kernel. An average separation contains approximately 15% of lipids of which 4% to 10% is considered as the phosphatide fraction. The work of Channon and Foster (1934) has shown that the phosphatide fraction of germ is made up of phosphatidic acid (as Ca, Mg, and K salts), lecithin, and cephalin. These compounds occur, according to Channon and Foster, in the proportion of 4:4:1 when referred to the phosphorus content.

The unsaponifiable material from an average germ separation amounts to 4% to 5% of the oil, of which about 70% is a mixture of the saturated sterol, dihydrositosterol, and several doubly unsaturated sterols called α_1, α_2, α_3, β and γ sitosterol (Anderson, Shriner and Burr, 1926; Wallis and Fernholz, 1936; and Bernstein and Wallis, 1939). The material remaining after the separation of the sterols is a yellow oil containing among other polyenes the tocopherols which possess vitamin E activity and certain of which have been recently synthesized.

The fatty acids obtained after the removal of the unsaponifiable material from wheat germ have been identified as follows (Sullivan and Bailey, 1936):

Palmitic acid	11.76
Stearic acid	3.06
Lignoceric acid	1.18
α linolenic acid	1.83
β linolenic acid	1.72
α linolic acid	22.32
β linolic acid	29.99
Oleic acid	28.14

The saturated acid fraction amounting to 16% was composed largely of palmitic acid, while the unsaturated fraction contained over 52% of linolic acid.

The lipids of wheat flour contain more volatile fatty acids than those of wheat germ. As is the case with the wheat-germ lipids, the bulk of the saturated fraction is palmitic acid. The same unsaturated acids, oleic, linolic, and a small amount of linolenic are present, as in wheat germ, with linolic the predominating acid of the unsaturated series. Flour lipid contains a somewhat higher amount (5.5%) of unsaponifiable material than wheat-germ lipid with, however, only about one-half instead of 70% of the unsaponifiable fraction being precipitated by digitonin (Sullivan and Howe, 1938). Other separations of flour and feed do not vary widely in the distribution of the fatty acids present in the glycerides and vary only from 4% to 7% in the amount of unsaponifiable material.

Wheat, as is true of so many other plant products, has a certain amount of its total lipids bound with the proteins and carbohydrates in complexes such that the usual fat solvents, *i.e.* ether, acetone, and ethyl acetate, do not give complete extraction. Hence, to obtain the phosphatides and other fat-like compounds as well as the glycerides, a preliminary treatment with alcohol or ammonia must be used previous to extraction by ether. The difficulty in the removal and purification of plant phosphatides is well known to all workers in this field. It has been found extremely difficult to purify the phosphatides from either flour or the embryo. Practically all preparations contain some sugar (approximately 2%) even after repeated precipitations with acetone. With most milling separations the N : P ratio is always in excess of 1. The phosphatide preparations from wheat germ, however, show a N : P ratio of nearly 1 : 1 according to our results. Because of the high ratio of N : P (7 : 1) in the phosphatide separation from flour it is evident that there is either protein or some nitrogen-containing lipids other than lecithin or cephalin. Work in this laboratory, which we hope to publish shortly, has shown that the lipids from flour carry a nitrogen-containing substance with an S–S $\rightleftarrows$ S–H linkage and that this compound can be concentrated in the acetone insoluble fraction of the fat. The lipids from the embryo do not give the test for the S–S or S–H linkage. The compound in question has not as yet been obtained in a sufficient state of purity to be identified. Balls and Hale (1940) have recently published a preliminary report on this substance.

The Influence of the Lipids on the Keeping Quality of Wheat Products

Flour, especially low-grade flour, bran, and germ contain lipases which under proper conditions of moisture, temperature, and pH hydrolyze the glycerides forming fatty acids (Sullivan and Howe, 1933). The hydrolysis is much more rapid at the higher moisture and temperature levels. If flour is stored in a cool dry place it maintains its baking quality for several months. Storage for long periods, especially in warm humid climates, causes the flour to deteriorate and become unsatisfactory for bread-making purposes. Such a damaged flour exhibits a " short " gluten and dough—that is, a dough that lacks extensibility and tears easily. The handling characteristics of the dough, its gas retention, volume, flavor, and taste, all suffer considerably in bread made from such a flour.

A flour which has gone out of condition for this reason may be extracted with ethyl ether, the fatty acids and fat removed, and fresh-flour fat (extracted from fresh flour of the same grade with ether) added back in the amount originally present in flour with the result that the baking quality becomes normal. The ether-extracted, aged flour without the addition of the ether extract from a fresh flour gives poorer volume in many instances than the out-of-condition flour. It is interesting in this connection that although fat from a freshly milled flour will bring back to normal the ether-extracted sample, fat from fresh-wheat germ will not, although the latter improves the handling characteristics of the dough. Since the fatty acids of the glycerides are not significantly different in kind and amount in any of the various milling separations we have not as yet succeeded in explaining this observation.

Kosmin (1934, 1934a, 1935) has shown that unsaturated fatty acids influence the colloidal behavior of the gluten by thickening the gel. Saturated acids show no such effect. Resnitschenko and Popzowa (1934), in an extension of Kosmin's work found that the effect of unsaturated acids on gluten was not due to their acidity as such but to the presence of the group RCH : CHCOOM where $R =$ radical and M a metal or hydrogen. Both Kosmin and Resnitschenko and Popzowa judged the action of these compounds only by the feel of the hand-washed gluten.

Our own work (Sullivan, Near, and Foley, 1936) has shown that the unsaponifiable material from the lipids of the various separations and the simple triglycerides such as tripalmitin, tristearin, trimyristin, and triolein have little or no effect on the behavior of a patent flour as

shown by plasticity measurements and baking tests. The higher saturated fatty acids which may occur in small amounts in any wheat product as a result of hydrolysis of the triglycerides have only a very slight "shortening" effect on the flour. Unsaturated fatty acids such as oleic, linolic, and linolenic produce "short," tough, and brittle glutens when added to sound normal flour. These unsaturated acids, however, are only slightly detrimental to the baking quality of flour. The bread is not harmed as much as one would expect from the feeling of the gluten. Individual unsaturated fatty acids influence a flour in direct correlation with their increasing number of double bonds. Unsaturated fatty acids when exposed to oxygen have a marked damaging action on the baking quality of flour. The dough feels dead, loses its elasticity and breaks easily. The volume of the loaf is decreased considerably. The action of these oxidized acids when added to fresh flour is identical with that of extreme natural aging of flour. The unsaturated acids which result by progressive hydrolysis of glycerides and phosphatides on aging a flour do not do much harm to the baking quality until they become oxidized either by oxidases present in flour or by the oxygen of the air. The oxidized products are peroxides rather than hydroxy acids.

Sinclair and McCalla (1937) found that the volume of an aged, badly deteriorated flour could be brought back by 5% of fresh germ but not by the alcohol-ether extract of the germ. These investigators thought that this effect might be due to the more insoluble lipids of the germ. It is our belief that the beneficial effect of this fresh germ on the aged flour sample was due to the presence of glutathione which has been found in the fresh germ and which reduces the oxidized fat.

The byproducts of milling—bran, middlings, and germ—are subject to a similar hydrolysis of the lipids. On aging, particularly in a sealed closed container, the alcohol-ether extract decreases, the ether extract increases and the phosphorus and nitrogen contents of the extracts decrease. The changes in amount of total lipid (alcohol-ether extract), in acidity, and in phosphorus and nitrogen content of the extracts are very much less marked when the samples are stored at 4% moisture than when stored at 13% or above. The higher the moisture content the greater the enzymic action, as might be expected. Samples of wheat or its products which are stored in cotton bags show relatively less change in all of the above factors (Sullivan and Near, 1933).

Thus it can be seen that the more complete the removal of the bran, shorts, and germ (all of which are higher than the flour in lipids and enzymic activity), the less serious are the storage and transportation problems.

The Influence of the Lipids on the Baking Quality of Flour

As has already been mentioned, a flour whose fat has become oxidized will show poor baking characteristics as reflected in lowered elasticity of the dough, as well as in poorer volume, texture, and grain of the baked bread.

A few workers have found that the ether extraction of a flour improves it while others working on different types of flour have found a decided detrimental effect. It has been our experience that ether extraction injures the breadmaking value of a flour although the gluten is in no way harmed. The dough made from the extracted sample is much tougher and more rubbery as is also the crumb of the finished bread. The volume of the loaf is considerably lowered. These characteristics may be restored completely by adding the extracted fat to the ingredients used in baking. Germ fat, cottonseed oil, lard, and many other shortenings will not fully bring back the extracted flour. We have found nothing so far which equals the flour fat in bringing back to normal an extracted flour.

Lecithin is beneficial in giving a smoother feeling dough but otherwise, when added in small amounts to a sound normal flour, it has very little effect on the baking properties. Shortenings such as lard, butter, or hydrogenated vegetable fat are usually added to a bread dough in amounts of from 2% to 4%. Shortening enables the sponge to be broken up more easily, gives a smoother dough and a more tender crumb. However, satisfactory though not quite as good bread can be made without shortening. Baker and Mize (1939) have shown that doughs which contain no shortening or liquid fats may become porous, allowing the expanding gas to escape, while dough containing semisolid shortening can retain much of the gas until after starch swelling and gluten coagulation in the oven. The phosphatides as well as other lipids *naturally* present in flour present a special problem.

When gluten, consisting principally of gliadin and glutenin, is washed out from flour it contains over half of the lipids of the flour in intimate combination. The gluten must first be treated with alcohol in order to liberate the lipids since only a trace can be removed by direct extraction with ethyl ether or petroleum ether. From 5% to 10% of the weight of gluten is lipid material. Gluten owes its singular properties in part to the lipids which are either adsorbed or in complex formation with the proteins.

Dough, which is an extremely complex colloidal system, has been thought of as a meshwork of coiled protein fibrils with starch and water. Lipids because of their property of reducing surface tension are con-

sidered to be distributed at the interfaces of this meshwork (Working, 1924). Too close an adhesion of the protein strands is prevented by the lipids which give more slippage and greater extensibility to the dough.

Bungenberg de Jong (1938) has shown that a pronounced shifting of the maximum to the acid side in the turbidity pH curve of an alkaline gluten sol occurs on the addition of small amounts of lecithin.

Toughness of a flour is in some way associated with the lipids. While there is much too little known concerning the exact role of the lipids in determining dough characteristics, all of the available findings indicate that the lipids are an extremely important factor in the proper estimation and knowledge of gluten quality. It is upon the amount and characteristics of the gluten that the baking quality, in a large measure, depends.

It is to be hoped that future work will throw more light on this problem.

Literature Cited

Anderson, R. J., Shriner, R. L., and Burr, G. O.
1926 The phytosterols of wheat germ oil. J. Am. Chem. Soc., **48**: 2987–2996.

Baker, J. C., and Mize, M. D.
1939 Effect of temperature on dough properties. II. Cereal Chem. **16**: 682–695.

Balls, A. K., and Hale, W. S.
1940 A sulfur-bearing constituent of the petroleum ether extract of wheat flour. Cereal Chem. **17**: 243–245.

Bernstein, S., and Wallis, E. S.
1939 Studies in the sitosterol complex. The isolation of α_3 sitosterol. J. Am. Chem. Soc. **61**: 1903–1904.

Bungenberg de Jong, H. L.
1938 Proteins in the flour industry. Symposium on Proteins. Nederlandsche Chemische Vereeniging, Eindhoven, p. 160.

Channon, H. J., and Foster, C. H. M.
1934 The phosphatides of wheat germ. Biochem. J. **28**: 853–864.

Kozmin, N.
1934 Reifung der Weizenmehle und ihre biochemischen Grundlagen. Mühlenlab. **4**: 18–31.
1934a Kleberqualität und einige Faktoren, die diese beeinflussen. Mühlenlab. **4**: 109–116.
1935 The aging of wheat flour and the nature of this process. Cereal Chem. **12**: 165–171.

Resnitschenko, M. S., and Popzowa, A. Q.
1934 Über die Wirkung ungesattigter Fettsauren auf die Kolloide Beschaffenheit des Klebers. Mühlenlab. **4**: 57–62.

Sinclair, A. T., and McCalla, A. G.
1937 The influence of lipoids on the quality and keeping properties of flour. Chem. J. Research **C15**: 187–203.

Sullivan, B., and Bailey, C. H.
1936 The lipids of the wheat embryo. I. The fatty acids. J. Amer. Chem. Soc. **58**: 383–390. II. The unsaponifiable fraction. J. Am. Chem. Soc. **58**: 390–393.

—— and Howe, M.
1933 Lipases of wheat. I. J. Am. Chem. Soc. **55**: 320–324.
1938 Lipids of wheat flour. I. The petroleum ether extract. Cereal Chem. **15**: 716–720.

—— and Near, Cleo
1933 Change of crude lipides of wheat on storage. J. Ind. Eng. Chem. **25**: 100–103.
——, Near, Cleo, and Foley, Guy H.
1936 The role of the lipids in relation to flour quality. Cereal Chem. **13**: 318–331.
Wallis, E. S., and Fernholz, E.
1936 α-sitosterol. J. Am. Chem. Soc. **58**: 2446–2448.
Working, E. B.
1924 Lipoids, a factor influencing gluten quality. Cereal Chem. **1**: 153–158.

THE EFFECT ON DIASTATIC ACTIVITY OF SURFACE, PRESSURE, DIFFERENTIAL, AND TEMPERATURE OF THE REDUCTION ROLLS IN MILLING

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A great effort has been made during the last twenty years to standardize analytical and baking methods for testing wheat. There is a corresponding need with reference to the experimental milling test, which is to-day often carried out exactly as it was thirty years ago, when Willard and Swanson (1911) published the details of their method and equipment. The necessity was recognized by the Milling and Baking Section of the Sixth International Technical and Chemical Congress of Agricultural Industries held in Budapest (Hungary) in July, 1939. This section passed a resolution that the laboratory milling test should receive priority in discussions at the next congress to be held in Rome.

Factors Involved in Milling

A few of the following isolated factors have already been studied by various workers. They will probably have to be tested in conjunction with other factors, as there is a lot of overlapping in their effects.

I. *Break rolls:* diameter, corrugation (number and form), surface, degree of spirality, differential, speed, length of roll.

II. *Smooth rolls:* diameter, surface (hardness of material, microscopic structure), true running, differential, speed, pressure, temperature, length of roll.

III. *Product:* hardness (type of wheat, preparation), moisture, fineness or granulation, characteristics of starch (resistance to mechanical treatment), quality of gluten (resistance to mechanical treatment), temperature.

IV. *Air in milling room or mill:* temperature, relative humidity.

V. *Milling technique:* flow sheet, roll adjustment, sifting and purification.

The whole problem is complicated by the fact that the effects on diastatic activity and on gluten quality are often diametrically opposed to one another, as has been mentioned by Gründer (1935), Pulkki (1938), and others.

It is surprising that so little is to be found on the subject of the present paper, even in well-known milling books, such as those by Kosmin (1921), Kettenbach (1922), Miller (1923), Dedrick (1924), and Hopf (1938). Most publications have been concerned with the influence of smooth rolls on the granulation of flour, a subject which has recently been reviewed by Kent-Jones (1939).

Through different methods of casting, very different surface structures of milling rolls can be obtained. About the only published reference to this is that of Raym and Scharffenberg (1932),[1] and it is also mentioned by Hopf (1938). Geddes and Frisell (1935) used steel reduction rolls which had been hardened by carbonizing.

No reference was found to the actual pressures used in milling, but Leatherock, McGhee, and Giertz (1937) mention the probable effect of the severity of pressure in grinding.

The temperature plays an important part. With cold rolls Geddes and West (1930) always obtained higher yields and they advocate warming up the rolls, as also does Miller (1937). Ostwald and Steinbach (1928) were interested only in granulation as influenced by temperature in milling with a coffee mill. The different cold milling systems (*e.g.* Loizillon) do not seem to have been studied with regard to their influence on diastatic activity. Strangely enough Levinson (1935) states that roll temperature has no great practical influence on the temperature of the product under the rolls. He reckoned that the product was in contact with the rolls for only 1/350 second.

As far back as 1879 Brown and Heron found that mechanically injured starch granules are more easily attacked by diastase (reported by Karacsonyi and Bailey, 1930). Blish, Sandstedt, and Kneen (1938) showed that sound, unruptured granules (raw starch) are not attacked by beta-amylase at all. Alsberg and Griffing (1925) state that flours with many injured starch granules, which can be stained *e.g.* by Congo red, have much material, beta-amylose, dissolved out of the granules. Karacsonyi and Bailey (1930) believe that the increase in diastatic activity due to overgrinding, as measured by the Rumsey autolytic method, is not due to any stimulation of the diastase,

[1] The microphotos of roll surfaces published by the authors are not of the surface itself, but of a cut perpendicular to the surface.

but rather to an increase in accessibility of the starch to the diastase. It is significant that in spite of this increase, gas production was not substantially modified by overgrinding. Pascoe *et al.* (1930) found an increase in diastatic activity equal to 35% after regrinding flour in a ball mill for 20 hours. Recently it has been shown by Sandstedt *et al.* (1939) that the available starch fraction, termed amylodextrin, is also very important as a factor influencing the handling quality of a dough.

Pulkki (1938a) thinks that the starch cells of wheat are surrounded by a film or a thin layer of substance which is easily removable by means of mechanical treatment. The explanation of the British Flour Millers' Research Association, however, seems to be better. Kent-Jones (1939) reports on their work according to which starch cells are probably ruptured around the edges, and starches probably show different degrees of resistance to the mechanical attack during milling.[2]

Apparatus

Figure 1 shows the modified experimental mill used for most of the tests here reported. The rolls could be very quickly changed, which was of special interest in the case of tests done at different temperatures.

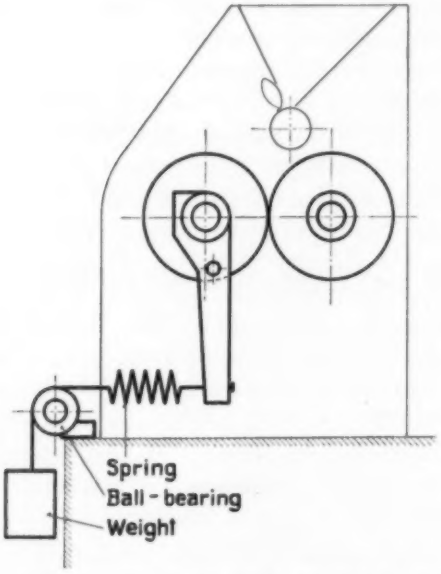

Fig. 1. Laboratory mill for working at a given pressure.

[2] Since the present paper was written C. R. Jones (Cereal Chem. 17: 133–169, 1940) has published results of outstanding importance on the work of the Association referred to. He differentiated between two factors. One of them, the "surface factor," is due to shearing or scraping of material from particle surfaces. This factor is influenced by roll surface and differential roll speed. On the other hand, there is the "internal factor," due to crushing or partial flattening of larger particles. Here the maltose figure depends on the size and hardness of the particles and on the roll pressure.

The main feature is the use of weights instead of springs to apply pressure. The strong spring shown in Figure 1 was included only in case some hard substance might get in between the rolls, but had no effect on the actual pressure. Exact pressures could be applied since, in addition to the weights, each of the two rolls was driven by a separate belt. With the use of gearing at the one end of the rolls—the usual practice in Europe—exact measurement of roll pressure can only be made by making allowance for the one-sided pressure caused by the gearing. For instance, when developing the Buhler roll-pressure applying and indicating device, by which any pressure can be set by a mere movement of the hand and kept constant, special attention had to be paid to this one-sided additional variable. In the mill (Fig. 1) the rolls had a diameter of 6 inches (150 mm.), the usual diameter used in experimental work; the length was 8 inches (200 mm.).

Three widely different roll surfaces were chosen. The term "polished" is used in this paper to designate a pair of new smooth rolls, whereas the rolls which are here termed "frosted" were treated with a sand blast. The degree of roughening depends of course on the hardness of the roll and the microscopical texture of the surface. The "scratched" rolls are those to which cross-cuts of 1 to 3 mm. length had been applied at 45° and 135° to the axe. The Brinell hardness was 555, which is very hard, but it is not implied that this is the optimum. The pressures used were 246, 465, 688, and 908 kg. per meter of roll length. These were actually obtained by adding the following weights to each end of the roll (Fig. 1): 7.7, 14.5, 21.5, and 28.4 kg. In commercial milling of the type of product used in our tests, a pressure of 600 to 700 kg./m. would be normal under European conditions. In some cases, on other products, pressures up to 1300 kg. have already been measured.

The speed of the fast roll was in all cases 340 rpm., which is slow for American conditions. Both in Europe and America 1 : 1.5 is a normal differential for the reduction rolls. In our tests 1 : 1 and 1 : 2.4 were also applied.

For the experiments on roll temperature effect the whole rolls were placed in a hot or a cold water bath for one hour before thorough drying and use in the mill. No arrangement was available for keeping the roll surface temperature constant.

The feed was kept constant, by setting the feed gate. The air in the milling room was 18°C. and had a relative humidity of 55%, except for Test 3. For one test the Buhler automatic laboratory mill was used.

Material and Methods

Most of these preliminary tests were done with coarse middlings of medium hardness going to the third reduction of a commercial mill, having a moisture content of 14.0%. In some cases flakes were produced, which were eliminated by gentle rubbing. In all cases the product was milled only once, so that the contact with the rolls would only be approximately 1/100 second. The maltose tests were made on the whole product.

With the autolytic saccharification in flour the limiting factor is the quantity of readily available starch present (Blish, Sandstedt, and Kneen, 1938), but—the same authors go on to say—autolytic flour "maltose values" represent approximately a 60% conversion of the available starch fraction.

Besides the Blish and Sandstedt ferricyanide maltose determination, the Berliner and Schmidt (1933) colorimetric maltose method, which is calibrated against the Berliner and Rüter (1928) titrimetric method, was used. Since the autolysis in this last case was only done with a 10% flour-water suspension for 30 minutes at 25°C. much lower values were obtained than by the method of Kent-Jones. The latter advocates a maltose figure between 1.8% and 2.3%, which would correspond to 1.0% and 1.4% by the other method. The colorimetric method is now done with a 20% flour-water suspension, which is digested for 60 minutes at 27°. Fifteen cc. of filtrate is boiled with 5 cc. of normal NaOH. Maltose as well as glucose and levulose are caramelized, but sucrose is not. The depth of yellow-brown color is correlated to the maltose figure. Because of its extreme simplicity this colorimetric method is widely applied in certain parts of Europe. At first a series of standard colored liquids in sealed tubes was used; now the comparison is usually made with a calibrated photoelectric cell (Berliner and Kranz, 1937). Schmidt (1938) reported on further aspects of the method.

The ash determinations were made at 600°C. by the direct ashing method. The temperatures of the stationary rolls were measured with a very fine thermocouple specially constructed to give a good contact with the surface.

Experimental

Test 1. Surface, differential, and pressure: For this test one pound of product was milled under each of the 36 different conditions. Temperature was not considered in this first test, which was carried out six years ago. The results are shown in Figure 2 and Table I. The figure shows that with a very smooth surface even a high differential and a high pressure yields only a low maltose figure, in spite of the

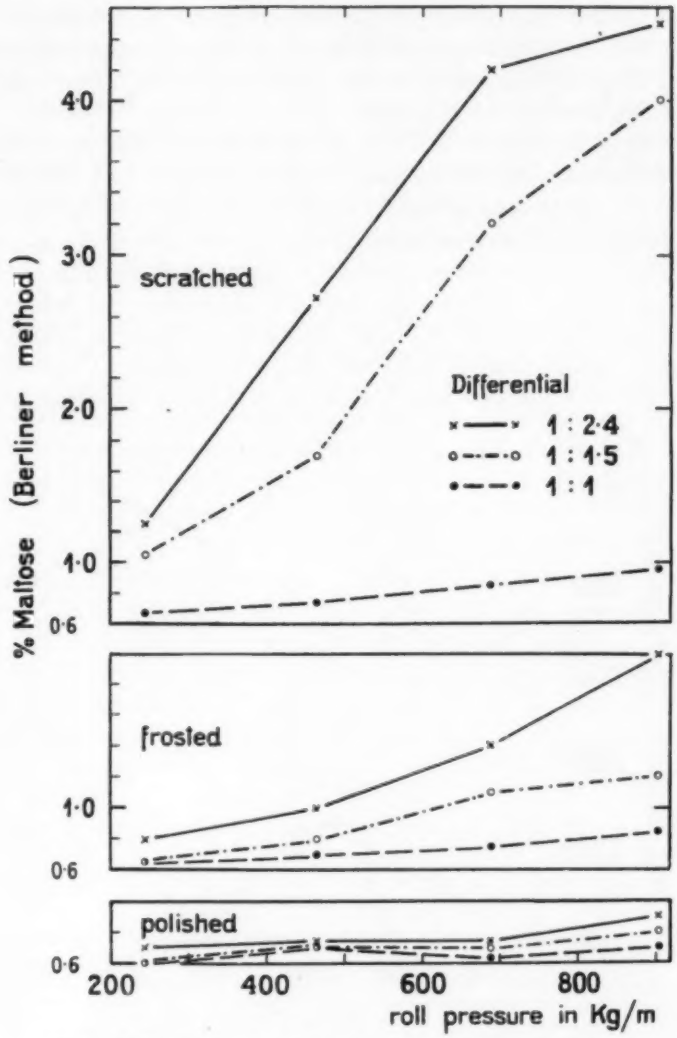

Fig. 2. Influence on maltose figure of surface, pressure, and differential.

TABLE I

INFLUENCE OF ROLL PRESSURE AND DIFFERENTIAL ON DIASTATIC ACTIVITY WITH THE "SCRATCHED" ROLL SURFACE

Data represent mg. maltose per 10 g. flour (Blish and Sandstedt method)

Differential	Roll pressure in kg./m.			
	246	465	688	908
1 : 1	120	—	150	—
1 : 1.5	—	201	244	—
1 : 2.4	198	240	—	312

fact that in this case a fairly high temperature was developed. Naturally it was the scratched surface which produced the highest maltose figures. With this surface, even with a normal differential and pressure, high values are obtained.

The increases shown in Table I and measured by the Blish and Sandstedt method are not so large in percentage as with the Berliner method. Whereas diastatic activity increased constantly, this cannot be attributed to greater fineness alone, because there is a maximum pressure for a product above which no greater fineness can be obtained.

Test 2. Surface and ash: This test was done with the Buhler automatic laboratory mill, with which the same sample of Manitoba 2 wheat prepared in exactly the same way was milled. The rolls were set to a definite distance in this case, on the three reduction streams

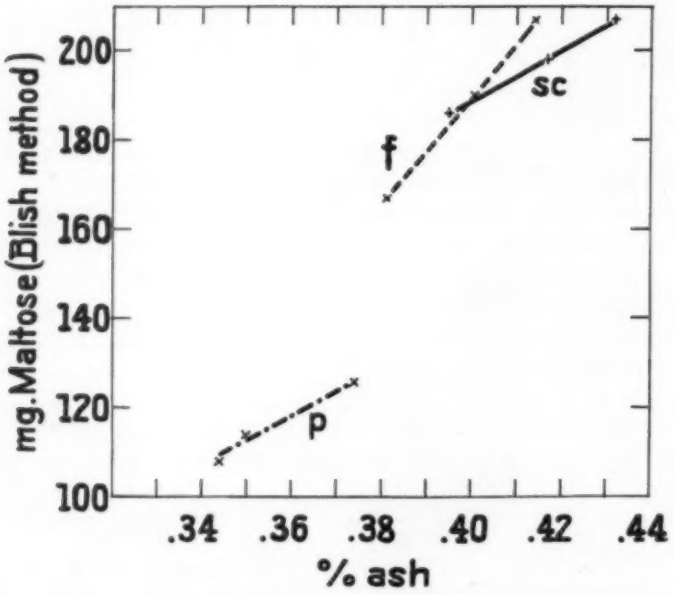

Fig. 3. Influence of roll surface on maltose figure and ash content (13.5% moisture basis). *sc* = scratched surface. *f* = frosted surface. *p* = polished surface. The lowest ash value of each curve is for first-reduction flour alone. The middle value for first- + second-reduction flour. The highest value is for a mixture of all three reduction flours.

to 0.07, 0.05, and 0.03 mm. Figure 3 shows the relationship of maltose to ash for the three different surfaces. The flour yields were:

Surface	Total yield in reduction flour
Scratched	49%
Frosted	46%
Polished	40%

The superiority of the rough surfaces for diastatic activity and yield is clearly seen and cannot be due only to the higher ash content; otherwise the curves marked *sc* and *f* would be in the prolongation of curve *p*.

Test 3. Roll temperature: The smoother the roll surface the more heat is developed during milling. After milling for one hour in the Buhler mill at a given setting (rolls not touching) and capacity the following temperatures were measured:

Scratched surface (cool, exact temperature not measured)
Frosted surface 40°
Polished surface 55°

The following test was done with the mill shown in Figure 1, using the frosted surface, 690 kg./m. and a differential of 1 : 1.5. The product used here had a moisture of 15.1% before milling, the room temperature was 15°–17° with an air humidity of 43%, and the barometer was 705 mm. Hg. In each case 2 kg. of middlings were milled. As abscissas of Figures 4 and 5 the average temperatures of both rolls before and after milling were taken. Figure 4 shows that under 45° the rolls had a tendency to warm up, whereas over that temperature a cooling down took place. Flour temperature increased as a linear function of roll temperature between 14° and 41°. This produced a loss in moisture up to 2%, although the product was only milled once.

The increase in maltose—as measured by the Blish method (Fig. 5)—was 42% from 6° to 20° and 32% from 20° to 78°, from 30° onwards the increase being fairly linear.

Discussion

Table II summarizes the results obtained. The effect of tempera-

TABLE II

SUMMARY OF THE RESULTS OBTAINED

Maltose figure	Pressure	Differential	Surface	Roll temperature
High	Medium	Medium	Rough	—
"	High	High	Medium	—
"	Medium	Medium	Medium	High
Low	High	None	Rough	—
"	High	High	Smooth	—[1]

[1] Under these conditions roll temperature would be fairly high.

ture alone is not yet quite clear. There may be an activating influence on one of the enzymes, but the greatest effect is no doubt on the starch.

Most authors have given figures showing that the diastatic activity of flour from laboratory-milled wheat is only about 65% of that ob-

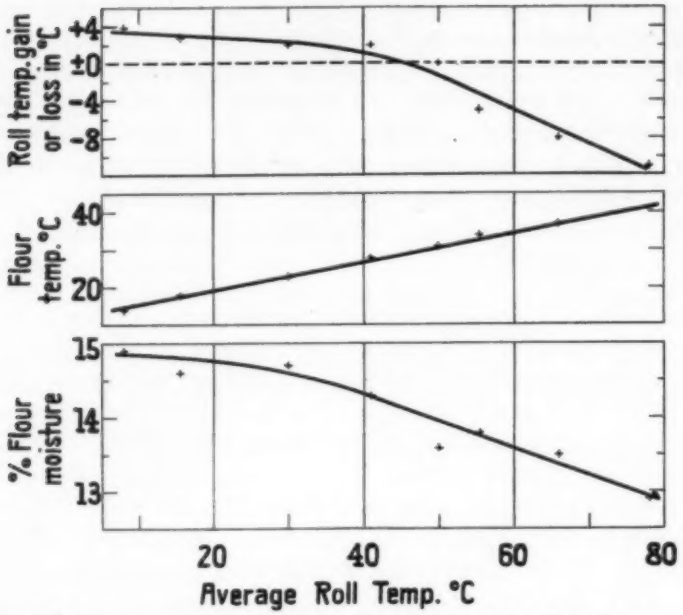

Fig. 4. Roll-temperature difference between beginning and end of milling (above), average roll temperature as a function of flour temperature (middle), and of flour moisture (below).

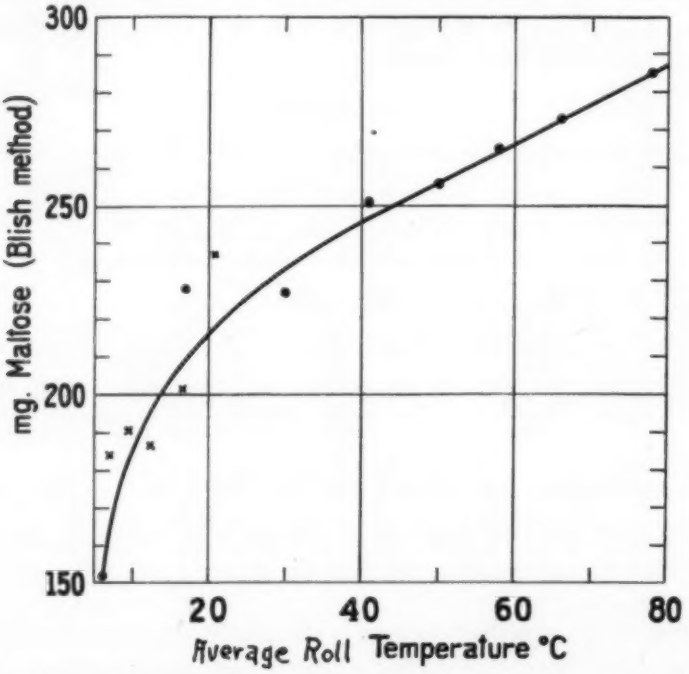

Fig. 5. Influence of average roll temperature on diastatic activity. Crosses imply duplicate and dots quadruple sugar determinations. "Frosted" surface.

tained by commercial milling. The curves of Anderson (1938), who compared commercial flour to flour from the Buhler laboratory mill, show that in some cases there is good agreement in diastatic activity and gas production; in others again the usual lower values for the laboratory flour occur. In view of the results discussed here and of the fact that in some American commercial mills reduction rolls are allowed to run very warm, it is believed that temperature differences at least explain part of the discrepancy found between commercially and laboratory milled flour. It may be that in the case of large mills which maintain cool reduction rolls the correlation of the maltose figure with that of experimentally milled flour is better than average.

To the effect of temperature must be added that of surface structure of rolls. The present study confirms the statement by the author (1938) that laboratory milled flours can be obtained with a normal diastatic activity and that it is a question of choice of roll surface. However other factors, such as gluten quality, do not permit the use of the roughest possible surface.

Summary

The main factors involved in milling have been enumerated. A laboratory mill has been described, which permits the accurate measurement of roll pressure. The normal pressure used in the milling of coarse middlings is about 600–700 kg. per meter roll length.

Middlings were milled using three different roll surfaces, roll pressures between 250 and 900 kg./m., three different differentials and roll temperatures between 6° and 78°C.

In the absence of differential or with a polished surface no significant increase in diastatic activity was caused by milling, whatever the pressure and surface in the first case, or the pressure and differential in the second case.

In the presence of a differential, the rougher the surface and the higher the roll pressure the greater the increase in diastatic activity.

The smoother the reduction rolls, even if they do not touch each other, the more heat they develop in milling. Roll temperature below 20°C. had more effect on diastatic activity per degree increase than above that temperature. From about 30°C. to 80°C. the increase in maltose figure seemed to be a linear function. Although the product was only in contact with the rolls for approximately 1/100 second the effect of milling on diastatic activity was very noticeable.

The higher maltose figures of commercially milled flours, as compared with experimentally milled flours, doubtless result in part at least from the higher roll temperatures involved in the former process.

Acknowledgment

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Literature Cited

Alsberg, C. L., and Griffing, E. P.
1925 Effect of fine grinding upon flour. Cereal Chem. **2**: 325–344.
Anderson, J. E.
1938 The Buhler mill. Northwestern Miller, Prod. No., Oct. 12, pp. 34–36.
Berliner, E., and Kranz, W.
1937 Kolorimetrische Messungen als Schnell- und Mikrobestimmungen. Mühlenlab. **7**: 89–94.
—— and Rüter, R.
1928 Titrimetrische und polarimetrische Zuckerbestimmung. Z. ges. Mühlenw. **5**: 116–120.
—— and Schmidt, E.
1933 Über Biuretreaktion, Trommersche Zuckerprobe und eine neue, einfache kolorimetrische Zuckerbestimmung. Mühlenlab. **3**: 187–192.
Blish, M. J., Sandstedt, R. M., and Kneen, E.
1938 The cereal amylases with reference to flour and malt behavior. Cereal Chem. **15**: 629–657.
Dedrick, B. W.
1924 Practical milling. National Miller, Chicago.
Geddes, W. F., and Frisell, B.
1935 An experimental flour mill for 100-gram wheat samples. Cereal Chem. **12**: 691–695.
—— and West, H. E.
1930 A study of the reliability of the experimental milling test. Sci. Agr. **10**: 333–343.
Gründer, W.
1935 Der Einfluss der Korngrösse auf Kleber- und Triebeigenschaften bei Weizenmehlen. Mühlenlab. **5**: 17–26.
Hopf, L.
1938 Taschenbuch für Müllerei und Mühlenbau (2nd ed.). Verlag "die Mühle," Leipzig.
Karacsonyi, L. P., and Bailey, C. H.
1930 Relation of the overgrinding of flour to dough fermentation. Cereal Chem. **7**: 571–587.
Kent-Jones, D. W.
1939 Modern cereal chemistry (3rd ed.). Northern Publishing Co., Ltd., Liverpool.
Kettenbach, F.
1922 Müllerei und Mühlenbau. Verlag Degener, Leipzig.
Kosmin, P. A.
1921 Flour milling. Routledge and Son, London.
Leatherock, L. E., McGhee, H. W., and Giertz, J. W.
1937 Diastatic activity in connection with flour mill control. Cereal Chem. **14**: 161–181.
Levinson, — —.
1935 Etude sur la température des produits qui sortent des appareils à cylindres. Bull. école française de meunerie, No. 44 and 45.
Miller E. S.
1923 Observations on milling. National Miller, Chicago.
1937 The purpose of experimental milling. Bull. Assoc. Operative millers p. 838.
Ostwald, W., and Steinbach, W.
1928 Beiträge zur Kenntnis der Mahlgänge. Z. ges. Getr. **15**: 72–77. Also Koll. Zeit. **43**: No. 3.
Pascoe, T. A., Gortner, R. A., and Sherwood, R. C.
1930 Some comparisons between commercially and experimentally milled flours. Cereal Chem. **7**: 195–221.
Pulkki, L. H.
1938 Über die Einwirkung des Ausmahlungsgrades und der Vermahlungsmethodik auf die Mehlbeschaffenheit. Mühlenlab. **8**: 49–52.

1938a Particle size in relation to flour characteristics and starch cells of wheat. Cereal Chem. **15**: 749–765.

Raym, W., and Scharffenberg, E.

1932 Härte und Griffigkeit bei Hartgusswalzen und sonstigen Mahlkörpern. Z. ges. Getr. und Mühlenw. **19**: 81–84.

Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.

1939 Starch in relation to some baking properties of flour. Cereal Chem. **16**: 780–792.

Schmidt, E.

1938 Eine kolorimetrische Zuckerbestimmung. Mühlenlab. **8**: 121–132.

Willard, J. T., and Swanson, C. O.

1911 Milling tests of wheat and baking tests of flour. Kansas State College Bul. 177.

Ziegler, E.

1938 The use of a purifier in experimental milling. Cereal Chem. **15**: 663–671.

FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. I. EFFECTS OF AUTOLYSIS ON THE CHARACTERISTICS OF DOUGH MIXER CURVES[1]

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(Read at the Annual Meeting, May 1940)

The variability of curves made by a recording dough mixer from different flours, even from the same variety, has been shown by Swanson (1939). The determination of factors that may cause curves to vary is the object of the present series of studies.

Dough has three principal physical properties: plasticity, elasticity, and viscosity. These properties merge into each other, and, as a result of this merging, each characteristic cannot be isolated to permit independent study. Because of this, the experimental measures of the physical properties of dough do not have the same definiteness as if each one of these main properties could be measured by itself and uninfluenced by the others. The characteristics of a dough-mixer curve are the resultant of the changing plastic, elastic, and viscous properties of the dough while being mixed. During that period of mixing which is represented by the ascent of the curve, the water is brought into contact with and is adsorbed by the starch granules and the protein which forms the gluten. During this period, because of the stretching, folding, and restretching action of the moving pins in the mixer, the gluten strands become oriented into a more or less parallel pattern and this continuously developing arrangement increases the resistance to the movement of the pins through the

[1] Contribution No. 68, Department of Milling Industry.

dough because of increase in elasticity and viscosity. The spreading of the water films over the starch granules and adsorption on the granules are the chief causes of the development of plastic properties. Starch does not contribute to the elasticity; this inheres in the gluten strands.

The viscous properties are mainly inherent in the water films which cover both the starch granules and the gluten strands. Maximum resistance to the movement of the pins through the dough occurs when there is the greatest parallel arrangement of the gluten strands in the dough and before the strands start to disintegrate. The curve reaches the top at the stage of maximum resistance. The rate of increase in resistance and the duration of maximum resistance, giving sharp, rounded, or flat-top curves, is a variety characteristic. When the resistance decreases, the curves start on the down slope. The steepness of the down slope depends upon the rate of decrease in resistance, and this also is a variety characteristic.

These characteristics (rate of increase in resistance, duration, and rate of decrease) are related to inheritance and can thus be used in the evaluation of varieties. The curves are also influenced by the amount of water used in mixing the dough, by the protein content, and by the adjustments on the mixer. Hence, all these must be carefully considered in the interpretation of the curves.

The cause of the decrease in resistance is probably the disintegration of the gluten strands. Hale (1939) presents evidence that "proteinase" is present in wheat flour and that it is an enzyme of the papain type. Markley and Bailey (1938) cite statements which indicate that wheat germ, glutathione, water extracts of yeast, wheat malt, and proteolytic enzymes increase the mobility, that is, decrease the resistance. Cysteine accelerates the rate of decrease, while bromate retards it. Overmixing the dough past the point of maximum resistance gives a measure of mechanical damage to the gluten structure. They also indicate that the effect of wheat malt in increasing the mobility is due almost entirely to alpha-amylase rather than proteases and that it is possible that in some flours the only force active in increasing the mobility upon prolonged mixing is that inherent in the starch. Markley (1937) has shown the thixotropic nature of the starch-water systems. Skovholt and Bailey (1935) found that nonprecipitable nitrogen increased with the extent of mixing and if proteases were added this was much larger. However, the increase in precipitable nitrogen was too small to be commensurate with the physical breakdown of the dough. The changes in physical properties are thus shown to be due to both chemical and physical forces.

Cairns and Bailey (1928) digested a mixture of 25 g. of flour and 100 cc. of toluene water. After five hours of digestion of first middlings flour + 0.1 g. of trypsin, the decrease in viscosity was from 125, control, to 12 W&T°. Without added protease the change after 24 hours of digestion of straight winter wheat flour was from 145, control, to 40 W&T°. When 3% of sprouted wheat flour was present the decrease was from 121 to 35 W&T°. Thus there are substances in the flour itself which decrease the viscosity, but the rate is much less than when proteases are present.

Some random curves made in this laboratory showed that flour-water doughs undergo changes after mixing and incubation, and that these changes are reflected in the characteristics of the curves made on the incubated doughs. Further, the extent of these changes becomes greater with the duration of the time of incubation. The changes found are characterized by the resistance to mixing, rapidly reaching a maximum and a greater rate of decrease in resistance. These changes might be caused by the action of proteases on gluten, by liberating phosphatides which would decrease viscosity of the water films that form a continuous system through the dough, by the increase in the amount of free water in this system. Scott Blair (1938) states: "Flour suspensions lose viscosity on standing and so do doughs. . . . it seems that the protein takes up more water than it can afterwards hold. As it gradually gives up this water, the starch gains water, but does not immediately immobilize it in the same way as does the proteins and the viscosity falls. It is for this reason that doughs soften during fermentation."

There are, thus, several ideas as to the causes of the changes which occur in the flour-water doughs upon standing and which are shown in the characteristics of curves made on such doughs. One theory is that the changes are due to enzymes and the other is that they are due to a type of syneresis which would increase the freedom of flow of dough substances on each other. If the changes are due mostly to enzymes, then they should be influenced by inhibitors.

There is also the possibility that the decrease in resistance is due to mechanical tearing of the gluten strands as soon as the remixing starts on the incubated doughs. It is also probable that several of these causes are operative at the same time, making the solution of the problem more difficult.

Experimental Procedure

The general procedure in these experiments was to mix the flour-water doughs until the water was incorporated, remove the dough from the mixer, place it in a small dish, cover and place in a cabinet

controlled at 30°C. This treatment in the cabinet subjects the dough to what may be called autolysis.[2] After predetermined periods of incubation, the dough was replaced in the bowl of the National Swanson-Working recording mixer[3] and the curves obtained. This mixer requires only 35 g. of flour for each curve. Optimum absorption and a constant machine setting were used. The periods used at first for incubation were ¼, ½, 1, 2, 3, and 4 hours, but it was soon found that periods longer than two hours served no useful purpose. Several modifications or additions to this procedure were used which will be explained in connection with the several experiments. The absorption was so controlled that the consistency or "feel" of the dough was very similar to that of doughs used in baking. Not all curves obtained are presented in the figures which are given, but a selection was made of those curves which best illustrated the changes in the dough in connection with a particular experiment. Control curves were obtained for each important group.

The flours selected for this study were from Tenmarq, Turkey, and Chiefkan wheats and were chosen because it was known that their doughs had very pronounced differences in physical properties and hence would give curves of varying characteristics. The moisture, protein, and ash (not corrected for moisture) on these flours were as follows:

	Moisture, %	Protein, %	Ash, %
Tenmarq	12.62	12.81	.400
Turkey	13.14	13.05	.448
Chiefkan	13.61	12.80	.442

Effects of Autolysis on Curves

The control curves and the curves obtained after ½, 1, and 2 hours of incubation are shown in Figure 1. Curves were also obtained after 3 and 4 hours of autolysis and were very similar to those obtained after 2 hours. Considerable changes had taken place in the ¼-hour period and the ¼-hour curves differed from the ½-hour curves only in showing less amount of change. It is evident that the greatest amount of the change took place in the first half hour and that among the varieties, Chiefkan shows the greatest amount of change, Tenmarq the least, and Turkey intermediate. The three curves in the bottom row were included here to show the effect on Turkey flour of 12 mg. of papain added to the water at the time of the first mixing. These

[2] Autolysis is used here to designate the process resulting in the automatic disintegration of substances such as gluten or starch similarly to what happens when the presence of enzymes is known.

[3] The movement of the pen and chart paper in this mixer is such that the curves resemble those made by the Swanson-Working larger machine (Swanson, 1939). For a description of this machine see Cereal Chemistry 10: 11–21.

curves were made after the doughs had been incubated for 5, 10, and 15 minutes, respectively.

The curves from the autolyzed doughs differ from the control in these respects: The pen swung almost to the top of the chart at once, and this was caused by the plasticity yield value when the pins started moving through the dough. The height of this swing cannot be used

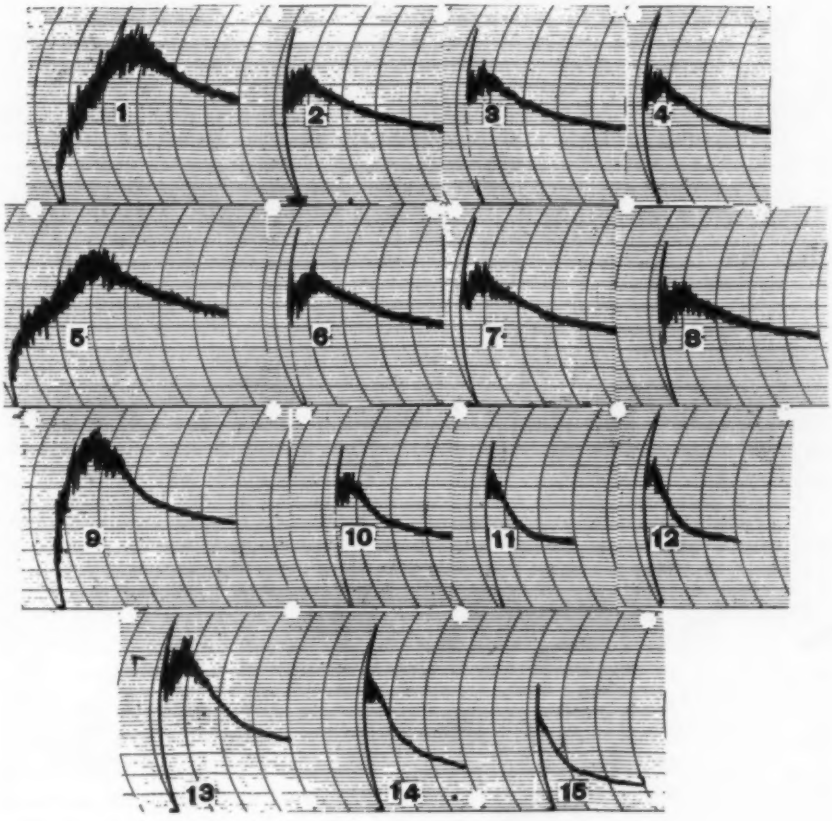

Fig. 1. Effect of autolysis.

(Figures in parentheses refer to the curve numbers.)

Top row, Turkey	(1) Control	(2) ½ hr.	(3) 1 hr.	(4) 2 hrs.
Second row, Tenmarq	(5) Control	(6) ½ hr.	(7) 1 hr.	(8) 2 hrs.
Third row, Chiefkan	(9) Control	(10) ½ hr.	(11) 1 hr.	(12) 2 hrs.
Bottom row, Turkey+papain	(13) 5 min.	(14) 10 min.	(15) 15 min.	

as a comparative measure of yield value because of the inevitable variation in handling when the autolyzed doughs are replaced in the bowl. After the first upward swing the width of the curve assumes the maximum and for Turkey and Tenmarq there is a short upturn, but not for Chiefkan. The rate of decrease in resistance is greater

for the autolyzed doughs than for the controls, especially for Chiefkan. The curves showing the effect of papain are similar to the others with the exception that the change was much greater. The papain produced in five minutes as great an effect on Turkey as autolysis for one-half hour, and in ten minutes the effect was as great on Turkey as autolysis for one hour on Chiefkan.

Effects of Varying Protein Content on Autolysis

The three flours used for the curves in Figure 1 had nearly the same protein content. Would the effects of autolysis be similar on high and low protein flours?

From wheat-variety testing experiments, there were on hand flours from several varieties which varied in protein content. Five flours from Turkey varied in protein from 9.5% to 15.6%; four from Michigan Wonder varied from 10.3% to 14.0%; five from Chiefkan varied from 10.6% to 15.4%; and five from Clarkan varied from 9.9% to 15.4%. Curves were obtained on all these flours both before and after one hour's incubation. From these curves there are shown in Figure 2 those from the highest and lowest protein samples in Turkey and Michigan Wonder and also the curves from the lowest, medium, and highest protein samples from Chiefkan and Clarkan. The check curves are placed in the first and third rows, and the curves from the autolyzed doughs in the second and fourth rows.

The effects of the protein content on the curve characteristics are striking. The tops of the curves from the high-protein flours are much higher than those from the low-protein flours. The protein content does not influence the time required for the curve to reach the top, and this makes the angle of the ascending and descending slopes much greater in the curves from the high-protein flours. The effects of autolysis are relatively the same on the low as on the high-protein flours. Chiefkan and Clarkan show a much greater increase in mobility than Turkey or Michigan Wonder. The conclusion from the curves shown in Figure 2 is that although the protein content has a great influence on the curve characteristics, it is not in itself a factor in the autolysis of the flour and water doughs.

Effects of $KBrO_3$ on Autolysis

The first thought might be that the changes which take place during incubation are caused by proteolytic enzymes. Their activity would weaken the gluten strands and when the dough is remixed the resistance would be greatly decreased as soon as the plastic yield was overcome. In overcoming the plastic yield, there is a tearing action of the weakened gluten strands. That proteases cause a decrease in

viscosity was shown by Cairns and Bailey (1928). If the cause of the large decreased resistance is due to proteases, then their action should be stopped or minimized by protease inhibitors such as $KBrO_3$. Flohil (1936) reports that $KBrO_3$ neutralized the effects of papain and malted wheat flour extract. In the wheat-meal-time-fermentation test it was found that $KBrO_3$ greatly increased the time from the

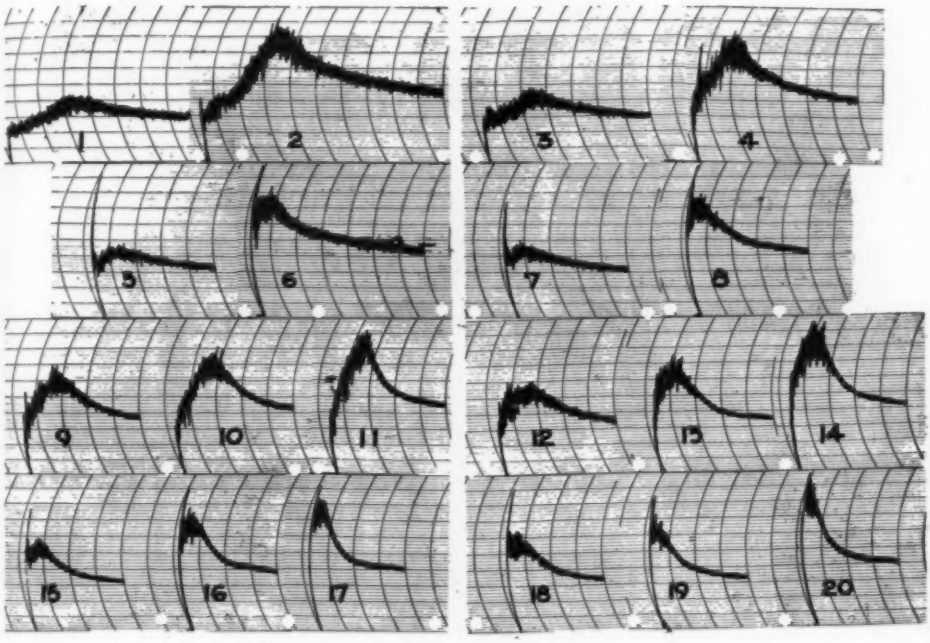

Fig. 2. Autolysis of flours of varying protein content.

	Turkey			Michigan Wonder		
Protein, %	9.5	15.6		10.3	14.0	
Checks	(1)	(2)		(3)	(4)	
Incubated	(5)	(6)		(7)	(8)	
	Chiefkan			Clarkan		
Protein, %	10.6	12.7	15.4	9.9	12.2	15.4
Checks	(9)	(10)	(11)	(12)	(13)	(14)
Incubated	(15)	(16)	(17)	(18)	(19)	(20)

moment the doughball was put on the water until a break was observed at the water-dough interface. It was found that proteases would also markedly decrease this time (Swanson and Dines, 1939).

The effects of using $KBrO_3$ are shown in Figure 3. The upper row of curves is from Turkey, the middle row, from Tenmarq, and the bottom row from Chiefkan. Each pair to the left was made using 16 mg. of $KBrO_3$ and each pair to the right with 100 mg. The first curve in each pair is the control; the second in each pair is the curve

obtained after one hour of autolysis. Longer and shorter periods were also used for autolysis, but the curves obtained after one hour illustrate what took place.

The presence of 16 mg. of $KBrO_3$ slightly increased the duration of the upturn in the curves obtained after autolysis, and with 100 mg. the upturn was even greater, especially for Turkey and Tenmarq. Part of the rise in the curves after autolysis may have been due to the salt effects, especially when 100 mg. were used. As is well known, certain salts, notably NaCl, have a stiffening effect on dough. That $KBrO_3$ has a marked effect on the dough during mixing has been shown by Baker and Mize (1937). Freilich and Frey (1939) found

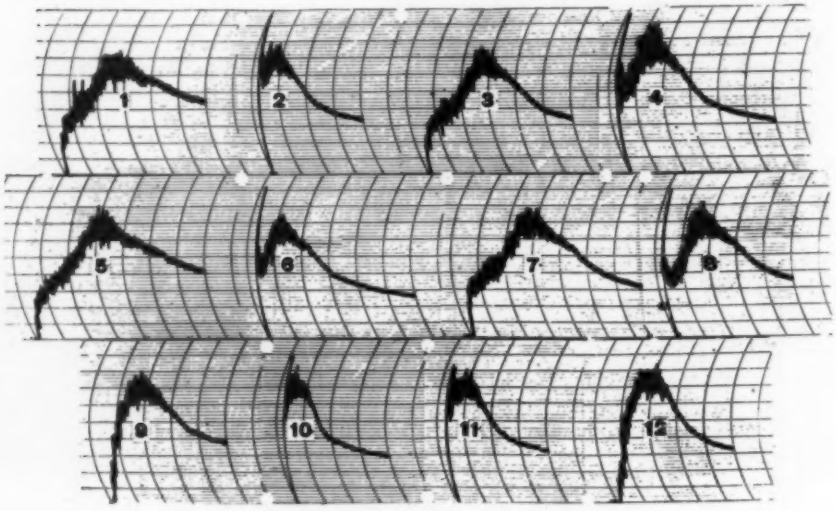

Fig. 3. Effects of $KBrO_3$.

	16 mg. $KBrO_3$		100 mg. $KBrO_3$	
Turkey	(1) Control	(2) 1 hr.	(3) Control	(4) 1 hr.
Tenmarq	(5) Control	(6) 1 hr.	(7) Control	(6) 1 hr.
Chiefkan	(9) Control	(10) 1 hr.	(11) 1 hr.[1]	(12) Control[1]

[1] These two have been reversed in position by mistake.

that increasing amounts of $KBrO_3$ gradually neutralized the effects of papain. Too large amounts, however, reduced the loaf volume. It thus becomes difficult to say whether there was any inhibiting effect from the $KBrO_3$.

Effects of NaCl and KBr

To learn how much the salt effects of $KBrO_3$ may have influenced the characteristics of the curves in Figure 3, NaCl and KBr were added to the water used in mixing the dough. The stiffening effect

of sodium chloride and similar salts on dough is probably due to the adsorbed ions holding around themselves shells of water molecules. This would decrease the amount of water in the free condition and thus diminish the ease of movement which is made possible by the water films on the gluten strands and on the starch granules and hence cause an increase in resistance. Such an effect would also be produced by $KBrO_3$, and this may partly account for the behavior of the curves from the doughs autolyzed with the 100 mg. of $KBrO_3$. This salt, however, has an additional action because of its oxidizing effects which the bromide, KBr, would not have. The curves obtained from using NaCl and KBr are shown in Figure 4. The left three

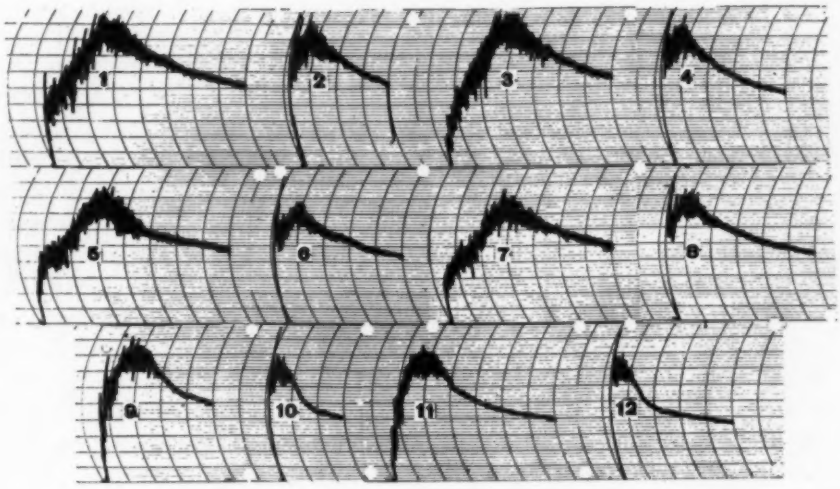

Fig. 4. Effects of NaCl and KBr.

	100 mg. NaCl		100 mg. KBr	
Turkey	(1) Control	(2) Autolyzed	(3) Control	(4) Autolyzed
Tenmarq	(5) Control	(6) Autolyzed	(7) Control	(8) Autolyzed
Chiefkan	(9) Control	(10) Autolyzed	(11) Control	(12) Autolyzed

pairs show the effect of NaCl and the right three pairs, the effect of KBr; the right one in each pair is the curve obtained after one hour of autolysis, and the left is the control containing the salt but obtained before autolysis.

The effects of each of the two salts are very similar, both before and after autolysis. Both the salts produce a stiffening effect shown by the increase in the heights of the curves. These two salts are not protease inhibitors; hence the rise in the curves after autolysis is due primarily to the effects of the salts restricting the mobility by the appropriation of water and hence decreasing the amount in the water

films. The similarities in the curves of Figures 3 and 4 indicate that the $KBrO_3$ did not have any protease-inhibiting effect.

Besides proteolysis there are other suggestions as to the cause of increased mobility in the autolyzed doughs. One is that it is perhaps due to a type of syneresis by which water is released from the protein, thus increasing the amount of free water. If the increased mobility is due to this cause, then the addition of starch, which would serve to appropriate this free water, should decrease the mobility. A few such trials were made, but with inconclusive results. Trials were also made to see if squeezing the autolyzed doughs between iron rollers would restore the viscosity, but the results of this were also inconclusive.

Summary Discussion

Doughs were mixed until the water was incorporated with the flour and then left in a cabinet controlled at 30°C. for various periods of time. They were then placed in the bowl of the recording dough mixer and the curves obtained. These curves from the autolyzed dough differed very much from the curves obtained immediately after the water came in contact with the flour. The plastic properties were more evident at first. The yield value was such that the pen swung nearly to the top of the chart at once, then swung back and forth, and there was only a small rise in the curve. This rise decreased in relation to the duration of the autolysis. The maximum resistance lasted only a short while when it rapidly assumed the minimum with a steep down slant of the curve. Considerable change had taken place in one-fourth hour, much more in one-half hour, and after two hours there was little further change. These changing characteristics were more evident in Chiefkan than in Turkey, and more so in Turkey than in Tenmarq.

The curves from autolyzed doughs were similar to those obtained with the use of papain. If this change upon autolysis was due to enzyme action, it was only slightly influenced by the presence of $KBrO_3$. There was some "salt effect" from $KBrO_3$ which was shown to be similar to the effects of KBr and NaCl.

The gradual softening of the dough becomes a factor when doughs are mixed for a long time. The continuous mixing would not have the same effect as remixing after the doughs have stood for some time as was the case with these autolyzed doughs. But since the effects of this standing was marked after 15 minutes and much more so after 30 minutes, it is easily possible that this change starts soon after the dough has reached maximum resistance in the mixing. This softening also becomes a factor when tests are made on doughs which are allowed to stand considerable periods before the tests are applied.

Literature Cited

Cairns, A. and Bailey, C. H.
1928 A study of the proteoclastic activity of flour. Cereal Chem. **5**: 79–104.
Baker, J. C., and Mize, M. D.
1937 Mixing doughs in vacuum and in the presence of various gases. Cereal Chem. **14**: 720–734.
Flohil, J. T.
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. Cereal Chem. **13**: 675–689.
Freilich, J., and Frey, C. N.
1939 Dough oxidation and mixing studies. I. The action of potassium bromate in dough. Cereal Chem. **16**: 485–494.
Hale, W. S.
1939 The proteinase in wheat flour. Cereal Chem. **16**: 695–702.
Markley, Max C.
1937 The colloidal behavior of flour doughs. I. The thixotropic nature of starch-water systems. Cereal Chem. **14**: 434–436.
—— and Bailey, C. H.
1938 The colloidal behavior of flour doughs. IV. The causes of the increase in mobility of the doughs upon prolonged mixing. Cereal Chem. **15**: 708–711.
Scott Blair, G. W.
1938 An introduction to industrial rheology (p. 69). P. Blakiston's Son & Co., Philadelphia.
Skovholt, O., and Bailey, C. H.
1935 The effect of mixing and fermentation upon the protein structure and the colloidal properties of doughs. Cereal Chem. **12**: 307–320.
Swanson, C. O.
1939 Variations in dough-development curves. Cereal Chem. **16**: 625–643.
—— and Dines, F. T.
1939 The wheat-meal-time-fermentation test. II. Effect of proteases, protease activators, and protease inhibitors. Cereal Chem. **16**: 168–177.

FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. II. EFFECTS OF ENZYMES ON CURVE CHARACTERISTICS [1]

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(Read at the Annual meeting, May 1940)

In a previous paper (Swanson, 1940b) it was shown that autolysis or merely incubating dough at 30°C. for various periods after it is mixed has a marked influence on the curve characteristics of this dough when it is remixed. The change was clearly noticeable after 15 minutes, and after one hour or more it was very marked. The curves obtained by remixing the dough after incubating at 30°C. for various periods resembled the curves obtained when papain had been added when the dough was first mixed and the curves run after only a few minutes' rest.

The main difference in procedure was in the length of the incubation

[1] Contribution No. 69, Department of Milling Industry.

period. While the end results were similar, the effects from papain were produced in a very much shorter time than when the water and flour doughs were autolyzed or incubated. The presence of $KBrO_3$ had little effect on the characteristics of the curves made on the autolyzed doughs, other than what may be termed salt effects. That this was a salt effect was shown more clearly by using KBr and NaCl for comparison. That the effects from autolysis were apparently not due to syneresis was indicated by the negative effects of adding starch or flour which would adsorb at least a part of the freed water in the autolyzed dough. Reworking and pressing the autolyzed dough through iron rolls also had a negative effect, indicating that the broken gluten strands could not be reunited by mechanical means.

In this paper the influences of the presence of enzymes on the curve characteristics are presented. Pepsin, trypsin, and papain were used as representatives of the proteases, diastase and taka-diastase as the amylases, and cysteine monohydrochloride as the protease activators. The effects of extracts of commercial wheat germ were used to compare with the action of the proteases. $KBrO_3$, as a representative of protease inhibitors, was also included in the study.

Procedure

The enzymes or other materials used were dissolved or suspended in the water so as to be thoroughly distributed while the dough was being first mixed. Each dough or curve was made on the National Swanson-Working recording mixer [2] from 35 g. of flour plus the needed water. Some curves were obtained from doughs without any rest period in order to obtain the effects of the enzymes during mixing, and others were made on doughs after resting or incubating for various periods so as to obtain the time effects.

Effects of the Three Proteases: Pepsin, Trypsin, and Papain

The three proteases were added in varying amounts starting with 2 mg. and increasing up to 20 mg., and the curves completed without any intervening rest period. The curves showed that amounts less than 12 mg. had no pronounced effects and amounts larger than this served no useful purpose since the curves were very similar to those obtained with the 12 mg. The curves obtained with each of the enzymes are shown in the upper row of Figure 1 for Turkey, and in the third row for Chiefkan. The first curve in each row is the check, the second curve shows the effect of pepsin, the third the effect of trypsin, and the fourth the effect of papain. Without incubation it is evident that on Turkey the least effects were produced by pepsin,

[2] Made by the National Manufacturing Company, Lincoln, Nebraska.

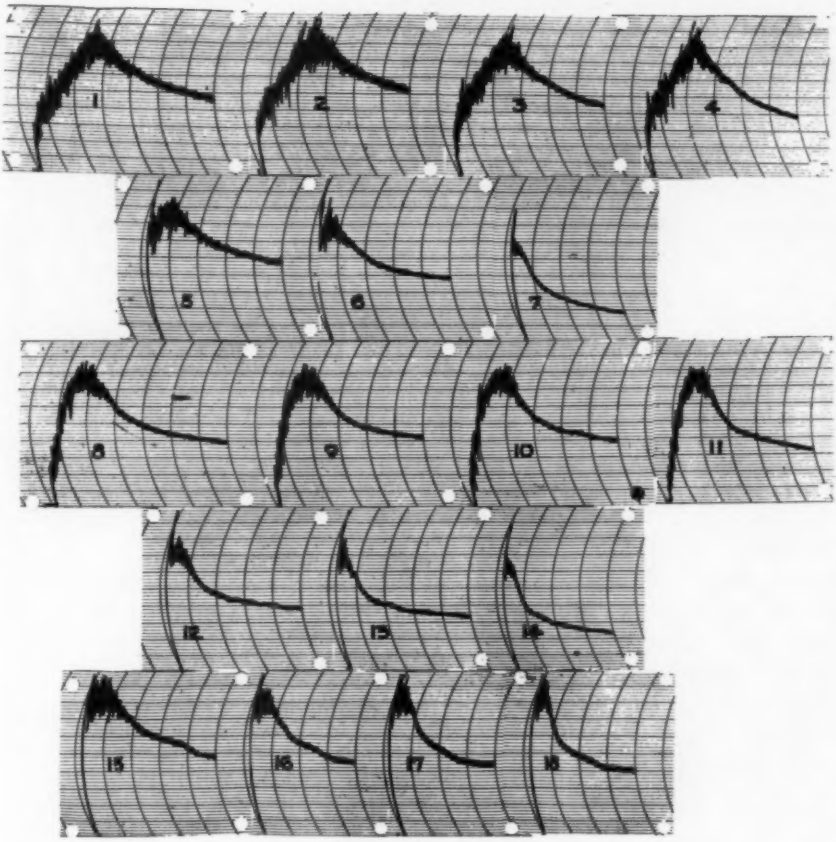

Fig. 1. Effects of the pepsin, trypsin, papain proteases, and extract of wheat germ on curve characteristics.

	No Incubation Period			
Turkey	(1) Check	(2) Pepsin	(3) Trypsin	(4) Papain
	Incubated 10 Minutes			
	(5) Pepsin	(6) Trypsin	(7) Papain	
	No Incubation Period			
Chiefkan	(8) Check	(9) Pepsin	(10) Trypsin	(11) Papain
	Incubated 10 Minutes			
	(12) Pepsin	(13) Trypsin	(14) Papain	
	Wheat Germ +10 Min. Incubation Period			
Turkey	(15) 5 cc.	(16) 10 cc.	(17) 15 cc.	(18) 20 cc.

the most marked by papain, and that trypsin was intermediate. On Chiefkan both pepsin and trypsin had greater effect than on Turkey, but for papain there was little difference in the effects on the two wheats.

Effects of Incubation with the Proteases

The fact that there was a noticeable effect from the enzyme while the dough was being mixed for the curve, indicated that the time for incubation need not be as long as for autolysis alone. Several trials showed that ten minutes of incubation gave the best results. The curves from the incubated doughs are shown in the second row of curves for Turkey and for Chiefkan in the fourth row in Figure 1. With the incubation it is evident that on Turkey, papain had the greatest effect, pepsin the least, and trypsin had more effect than pepsin. On Chiefkan, pepsin and trypsin had greater effect than on Turkey, while the effect of papain was of the same order. Balls and Hale (1936a) found that the addition of 50 mg. of papain or of glutathione to dough equivalent to a one-pound loaf practically liquefied the dough and the loaf did not rise at all.

Effects of the Water Extract of Wheat Germ

Commercial wheat germ was sifted to remove most of the bran particles. The germ was then ground in a hammer mill to pass a $\frac{1}{2}$-mm. sieve. This ground germ was soaked for several hours in water in the proportion of one to five. Thus five cc. of extract represented one gram of the germ. A clear extract was obtained by first filtering through linen cloth and then centrifuging. Portions of this clear but colored extract were used together with enough water to make doughs from the Turkey flours. After the preliminary mixing the doughs were incubated at 30°C. for ten minutes and the curves obtained therefrom are shown in the lowest row on Figure 1. It is evident that the effects on the curves are similar to those obtained with trypsin (6) when digesting for ten minutes. It thus appears that the effects are due to the proteases extracted from the germ or may be due to glutathione which is present in the germ. Sullivan, Howe, and Schmalz (1936) obtained marked modifications in the farinograms both from the water extract of wheat germ and from the extracted glutathione.

Effect of Cysteine Monohydrochloride

Balls and Hale (1936b) measured the rate of softening of flour paste by noting the time required for BB shot to sink below the surface. A paste of whole-wheat flour made with equal parts of flour and water saturated with CO_2 and incubated at 30°C. for 30 minutes presented a surface through which the shot took six minutes to sink. After the same flour had been bleached with chlorine, the time was increased to five hours. The addition of one mg. of cysteine per gram of dry flour reduced the time for the untreated flour to 20

seconds and to 80 seconds for the chlorine bleached flour. The authors state: "It is not a foregone conclusion that the liquefaction of the paste is due to a proteolytic ferment." They found also that kneading 40 g. of wet gluten with 100 mg. cysteine and incubating at 45°C. for 30 minutes liquefied the gluten to a milky white fluid that could be diluted with water. Thus the presence of the cysteine induces the peptization of the gluten and this in turn should increase the mobility of a dough as shown by the recording dough mixer curves.

The trial curves made with cysteine present showed that the rate of action was much more rapid and the amounts needed were much less than with papain. The effects on the curves when various amounts of cysteine were present in the dough are shown in the upper three rows of Figure 2. These curves were obtained respectively from Turkey, Tenmarq, and Chiefkan with 0, 3, 6, and 12 mg. of cysteine present and no intervening incubation period. It is evident that the mobility increases with the amounts of cysteine and that even 3 mg. produce very marked changes during the time that the dough is being mixed. It seems that part of the effect is immediate since the upslope of the curve is notably steeper than in the control curves.

Since 3 mg. of cysteine produced marked effects during the mixing, this amount was used with short incubation periods. It was found that a 10-minute period such as was used with the proteases reduced the dough to a sticky paste, too fluid to handle in the mixing bowl. Several trials showed that the mixing period and the rest period combined should not be more than 2 minutes. Turkey was mixed $\frac{3}{4}$ minute, Tenmarq 1 minute, the Chiefkan $\frac{1}{2}$ minute, and the rest periods were such as to make the total 2 minutes. The curves obtained on the three flours are given in the fourth row of Figure 2. The effects were almost the same on each of the three flours and were like that obtained with 12 mg. of papain after 10 minutes incubation (Curves 7 and 14, Figure 1).

Three curves from Turkey, Tenmarq, and Chiefkan are presented in the last row of Figure 2 to compare the effects of incubating the plain flour and water dough at 30°C. for several hours with the effects of cysteine acting for two minutes before beginning to run the curve. The Turkey and Chiefkan curves show about as great an effect from autolysis alone, as when cysteine was present, and the Tenmarq curve almost as much effect, but the effects from autolysis took several hours while only two minutes were required to produce the effects when cysteine was present.

Thus the same effects on the curves were produced by proteases, by cysteine, by autolysis, or by simply incubating the water and flour doughs for several hours. The main difference was in the time

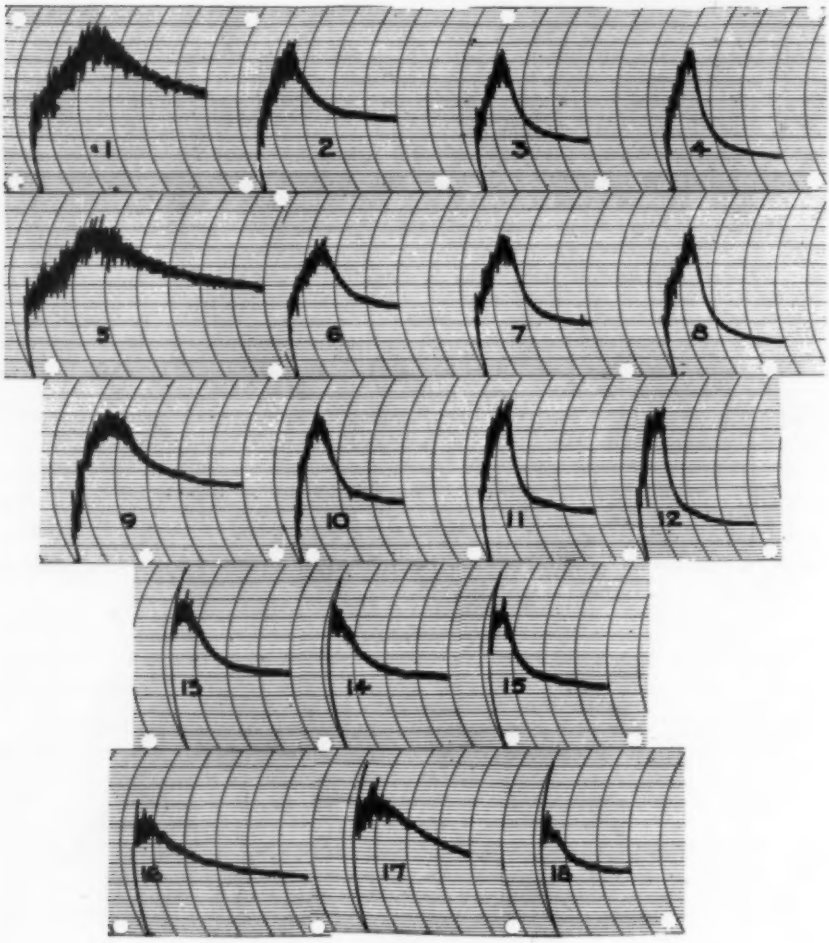

Fig. 2. Effects of cysteine.

No Incubation or Rest Period

	Milligrams cysteine			
	0	3	6	12
Turkey	(1)	(2)	(3)	(4)
Tenmarq	(5)	(6)	(7)	(8)
Chiefkan	(9)	(10)	(11)	(12)

Short Rest Period (3 mg. cysteine)

(13) Turkey (14) Tenmarq (15) Chiefkan

Several Hours' Autolysis

(16) Turkey (17) Tenmarq (18) Chiefkan

required. Cysteine produces the effect in the shortest time and with the least quantity. Papain requires five times as long as cysteine and autolysis alone requires from six to twelve times as long as papain. Balls and Hale (1936b) present the statement that the effect of cysteine

on gluten is not the activation of an enzyme but an action on a protein. The extreme speed of the reaction would favor this view. On the other hand, Jørgensen (1936) presented evidence to show that wheat flour contains powerful but inactive proteolytic enzymes. "Usually these enzymes are latent, but an activator such as glutathione which in itself does not possess proteolytic activity is able to stimulate the proteinases of wheat flour." Read and Haas (1937a) state that excessive dosages of a "proteinase" or some "proteinase" activator such as glutathione or cysteine hydrochloride will ruin the baking properties of any gluten. "A stiff dough may readily be reduced to the consistency of a thick soup."

Glutathione, according to Hopkins (1929), is a tripeptide, $C_{10}H_{17}N_3SO_6$, containing glycine, glutamic acid, and cysteine, thus indicating the possibilities of similarities in reaction between glutathione and the cysteine monohydrochloride. It is not illogical to suppose that cysteine may activate both the protein and the proteases. This double activation would, in part at least, help to account for the speed of the action of cysteine.

Effect of $KBrO_3$ on Pepsin and Papain

Potassium bromate is considered a protease inhibitor. The use of $KBrO_3$ in connection with the wheat-meal-time-fermentation test greatly increased the time from the moment the doughball was put into the water until disintegration could be observed at the dough-water interface (Swanson, 1940a). Flohil (1936) reports that 15 mg. of $KBrO_3$ neutralized the effects of 15 mg. of papain and that 30 mg. inhibited the effect of malted wheat flour extracts. Read and Haas (1937b) present data which fail to support the opinion "that the benefits commonly derived from the use of bromate in flour doughs result directly from its inhibitive action on the 'proteinase' contained in the flour." They found that bromate did not adversely affect the proteolytic power of malt, pepsin, trypsin, or taka-diastase when these products were allowed to act on gelatin. These authors have made further studies (Read and Haas, 1939) on the activation and inhibition of flour "proteinase." They report that $KBrO_3$ had a marked inhibition on papain but not on trypsin as measured by the liquefaction of gelatin.

The effect of $KBrO_3$ was obtained by adding 12, 24, 36, and 72 mg. portions in combination with either pepsin or papain each used in 12-mg. amounts, and the curves are given in Figure 3. The first row of curves shows the effect of the various amounts of $KBrO_3$ with pepsin, using no rest or incubation period. These curves are similar to curve (2) in the first row of Figure 1, where pepsin alone was used.

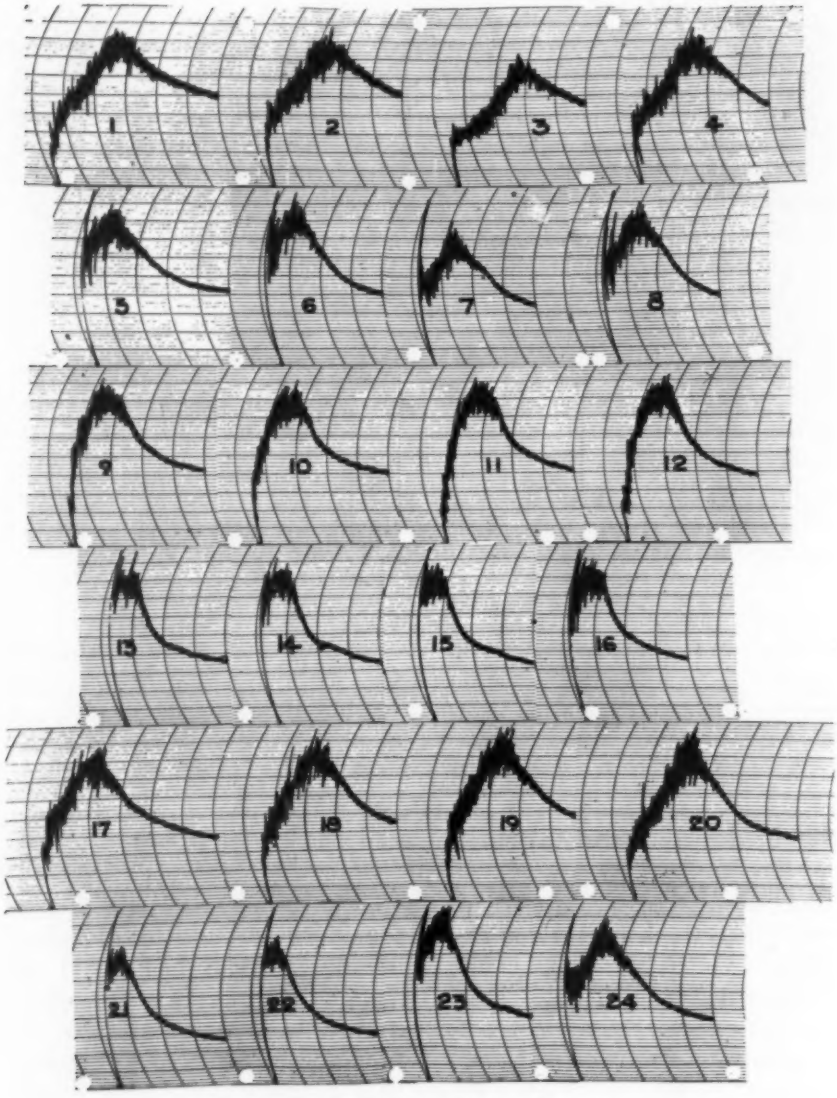

Fig. 3. Effects of $KBrO_3$ on pepsin and papain.

	Amounts of $KBrO_3$			
	12 mg.	24 mg.	36 mg.	72 mg.
	Turkey + 12 mg. Pepsin			
No incubation	(1)	(2)	(3)	(4)
10 min. incubation	(5)	(6)	(7)	(8)
	Chiefkan + 12 mg. Pepsin			
No incubation	(9)	(10)	(11)	(12)
10 min. incubation	(13)	(14)	(15)	(16)
	Turkey + 12 mg. Papain			
No incubation	(17)	(18)	(19)	(20)
10 min. incubation	(21)	(22)	(23)	(24)

Any deviation is evidently due to the salt effects of $KBrO_3$. The second row shows the additional effect of the 10-minute incubation period. The upslope of these curves is greater than those in the second row of Figure 1, showing that the presence of $KBrO_3$ had some effect on the curve characteristics. This difference is mostly in the first part of the curves which may be due to salt effects as shown in the preceding paper (Swanson, 1940b). The last part of these curves shows as much mobility as the curves in Figure 1 obtained in the absence of bromate. There were clearly no inhibiting effects on the increase of mobility in the last part of the curve.

The curves in the third and fourth rows of Figure 3 were made with Chiefkan flour under the same conditions as those of Turkey shown in the first and second rows. The effects of the pepsin and the $KBrO_3$ on Chiefkan are no different from the effects on Turkey except in so far as the Chiefkan curves are different from the Turkey curves without any additions.

The curves in the fifth and sixth rows were made from Turkey, but using papain instead of pepsin. The greatest effect in increased mobility was obtained with the smaller amounts of $KBrO_3$. When 36 and 72 mg. of $KBrO_3$ were used with no incubation period, there was a slower rate in the decrease of mobility, and with the incubation period there was considerable resistance shown on the upslope of the curve. The down slope, however, shows great increase in mobility; hence it is doubtful whether the $KBrO_3$ by itself exercised any effects on the proteases used.

Bleaching with chlorine was tried as an inhibitor to the action of papain and cysteine on the curve characteristics. Doughs were made with Turkey flour bleached both at the rate of $\frac{3}{4}$ and $1\frac{1}{2}$ ounces of chlorine per barrel and using 3 mg. of cysteine and 12 mg. of papain with subsequent treatment as in the preceding curves. No pronounced differences could be observed in the characteristics of the curves made from the bleached flours as compared with those made from the unbleached flours. This is contrary to the findings of Balls and Hale (1936b), who reported that the time of sinking of BB shot into the dough was greatly increased as a result of bleaching with chlorine.

Effects of Diastase and Taka-diastase

The diastases should influence the curve characteristics by their effects on the starch, which is present in the dough in six or seven times the amount of protein or gluten. This should be especially true of taka-diastase because of the alleged presence of the starch liquefying alpha-amylase. After several trials it was found that amounts larger than 12 mg. per 35 grams flour served no useful

purpose. The enzymes were added in water solution in order to be thoroughly incorporated with the dough. The curves obtained using the two diastases and the three flours are shown in Figure 4. The first curves in each row are the checks or those completed without any rest or incubation period. The middle curves in each row are those made after one hour's rest or incubation, and should show any enzyme effects exerted during the hour. The last curves in each row show the effect of one hour's incubation without the enzyme.

The first curves in each row are similar to those obtained with only flour and water. A comparison of the middle curves with the last curves in each row shows that autolysis in the absence of the diastases produced as great an effect as when the diastases were present. Further, the curves obtained with taka-diastase are but very little different from those produced with the diastase. Swanson (1940a) found that the diastases had no influence on the results of the wheat-meal-time-fermentation test while the proteases and protease activators, as well as the protease inhibitors, had a marked influence (Swanson and Dines, 1939). Read and Haas (1936), however, found that taka-diastase exhibited marked proteolytic activity. No evidence of this was found in these curves when taka-diastase was present.

Discussion

The effects of the proteases, pepsin, trypsin, and papain on curve characteristics have been presented in this study. The extract of commercial wheat germ was used for comparison. Cysteine monohydrochloride was used as a protease activator and $KBrO_3$ as a protease inhibitor. Diastase and taka-diastase were used to determine whether their action on starch had any influence on curve characteristics.

The proteases produced marked changes in the curves. These changes were similar to those produced by autolysis or incubating the flour and water dough one hour or more. The main difference was in time. The proteases produced in a 10-minute incubation period as much effect as one to several hours of incubation without the protease. Papain produced the greatest effect, pepsin the least, and trypsin produced an intermediate effect.

Two minutes of contact with cysteine, including both the mixing and resting time, produced as great a change as ten minutes with the proteases or several hours' incubation of the plain water-and-flour dough. From the previously quoted statements of Balls and Hale (1936b) as well as these results it would appear that cysteine activates both the protein and the proteases. Without such a supposition it is difficult to account for the rapid action of the cysteine.

As a representative protease inhibitor, $KBrO_3$ was used in 12, 24,

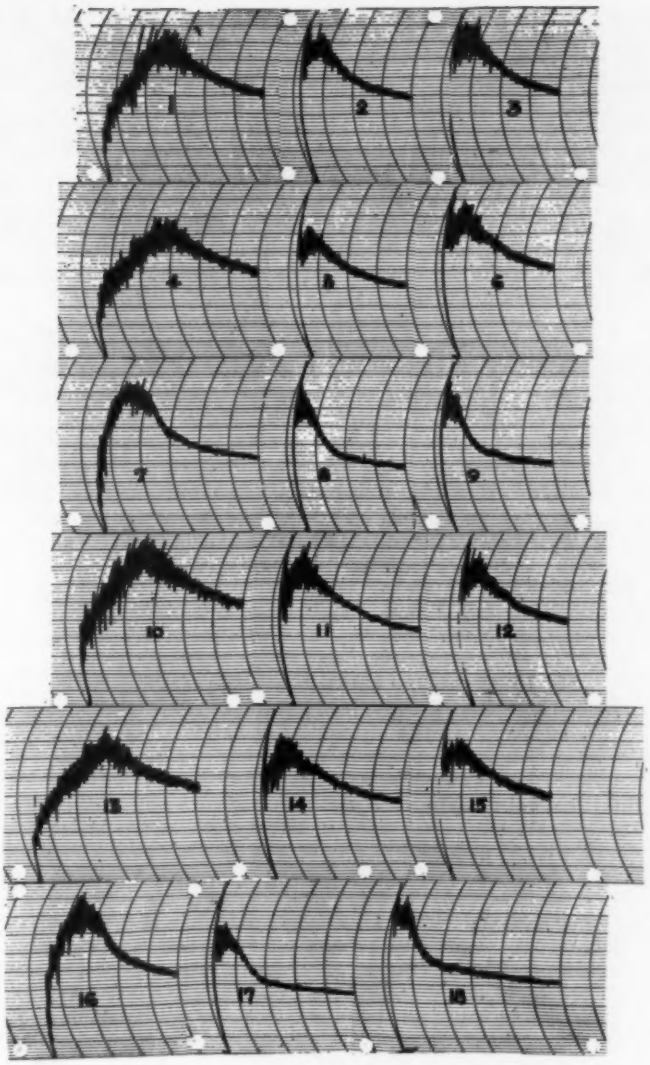

Fig. 4. Effects of diastase and taka-diastase.

	12 mg., no incubation	12 mg., 1 hr. incubation	No mg., 1 hr. incubation
	Diastase		
Turkey	(1)	(2)	(3)
Tenmarq	(4)	(5)	(6)
Chiefkan	(7)	(8)	(9)
	Taka-diastase		
Turkey	(10)	(11)	(12)
Tenmarq	(13)	(14)	(15)
Chiefkan	(16)	(17)	(18)

36, and 72 mg. amounts for each dough made with 35 g. of flour. From the characteristics of the curves it could not be shown that the $KBrO_3$ had any inhibiting effect. Salts have by themselves a stiffening effect on the gluten, and hence it is not possible to determine definitely whether the decrease in mobility was due to the inhibiting action of the $KBrO_3$ on the proteases or whether it was due to a salt effect.

The presence of diastase and taka-diastase produced no results essentially different from autolysis without these enzymes. Hence, their effects on starch were not noticeable in the curve characteristics.

Conclusion

The effects on curve characteristics of incubating flour-and-water doughs are very similar to the effects produced by proteases and the protease activator, cysteine, except as regards the time necessary for effects to become evident. From this it may be inferred that the influence of autolysis on curves can be attributed to proteases or protease activators.

Literature Cited

Balls, A. K., and Hale, W. S.
1936a Proteolytic enzymes of flour. Cereal Chem. **13**: 54–60.
1936b Further studies on the activity of proteinase in flour. Cereal Chem. **13**: 656–664.

Flohil, J. T.
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. Cereal Chem. **13**: 675–689.

Hopkins, F. G.
1929 On glutathione: a reinvestigation. J. Biol. Chem. **84**: 269.

Jørgensen, H.
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. Cereal Chem. **13**: 346–355.

Read, J. W., and Haas, L. W.
1936 Further studies on the baking quality of flour as affected by certain enzyme actions. Cereal Chem. **13**: 14–37.
1937a Baking quality of flour as affected by certain enzyme actions. III. Purified amylase and the relative proteolytic activity of amylolytic agents. Cereal Chem. **14**: 58–73.
1937b Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. Cereal Chem. **14**: 752–766.
1939 Studies on the baking quality of flour as affected by enzyme actions. VI. Further studies relating to the activation and the inhibition of flour proteinase. Cereal Chem. **16**: 60–70.

Sullivan, B., Howe, M., and Schmalz, F. D.
1936 On the presence of glutathione in wheat germ. Cereal Chem. **13**: 665–669.

Swanson, C. O.
1940a The wheat-meal-time-fermentation test. IV. Inhibitory and accelerating factors. Cereal Chem. **17**: 355–372.
1940b Factors which influence the physical properties of doughs. I. Effects of autolysis on the characteristics of dough mixer curves. Cereal Chem. **17**: 679–689.

Swanson, C. O., and Dines, F. T.
1939 The wheat-meal-time-fermentation test. II. Effects of proteases, protease activators, and protease inhibitors. Cereal Chem. **16**: 168–177.

A METHOD FOR THE FORMULIZATION OF FARINOGRAPH CURVES

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Considerable controversy has existed as to the best method for recording and interpreting curves produced by the Brabender farinograph and several different techniques have been suggested for the characterization of these data. However, in the course of a summer's work at the National Milling Company, Toledo, Ohio, during which farinograph curves were made for all incoming lots of grain, it was decided that the existing methods for the interpretation of these curves do not provide an adequate means for the expression of curve characteristics. The usual procedure is to consider only the time during which the upper line of the graph stays above a certain ordinate. This is very arbitrary and empirical and it yields only one item of data from the curve, whereas the curve may be capable of expressing much more. Furthermore, in accordance with the usual type of procedure, a true and complete picture of the curve would require many measurements.

This situation led to the thought that if the farinograph curve could be represented by a formula in which the consistency in Brabender units, as well as the mixing time, were given explicitly as functions of certain arbitrary constants, then all of the information contained in the curve would be available in compact mathematical form. This formula must, of course, be such a combination of consistency, time, and arbitrary constants that every farinograph curve could be reproduced by adjustments of the constants only.

With such a formula it is a simple mathematical manipulation to obtain from it any feature which the original curve contained, and the formula serves as a simple, compact evaluation of the original curve obtained from the farinograph. Also it is at least possible that correlations may be found between the constants of the formula and certain flour properties such as baking characteristics, protein, or other quality factors. With these aims in mind a method is herein proposed.

The Method

In obtaining a formula which is to fit the original farinograph curve, the formula must be such that when, using farinograph paper, values of time on the one hand are plotted against values for consistency on the other, a close approximation of the original curve will be reproduced.

Accordingly, by intuition and trial, the writer obtained the following formula which fits any farinograph curve, by adjustment of the arbitrary constants A, n, and c only:

$$K = At^n e^{-ct}$$

in which: t is the time in minutes,
K is the consistency in Brabender units,
e is the base of Napierian logarithms,
A, n, and c are the arbitrary constants adjusted to fit the curve in question.

It was found by numerous trials that this formula will accurately represent any farinograph curve from zero time up to a point considerably beyond gluten breakdown. Since the Brabender farinograph curves give no information at points far beyond the breaking point of the gluten, the formula fulfills its mission in representing the curve over its entire useful range.

Let us consider the theoretical aspects of the problem of fitting this formula to any given curve. Since there are three unknowns in the equation to be evaluated, namely the three arbitrary constants A, n, and c, it will be necessary to take three items of data from the original curve to solve for these unknowns. Thus, if we select three points on the curve in the region of interest, and substitute the coordinates of each of these in turn into the aforementioned equation, we will obtain three equations in which the only unknowns are the three arbitrary constants A, n, and c. It is well known that three independent equations of linear form with three unknowns may be solved to obtain the three unknowns by the simultaneous theory. However, by inspection it appears that the three equations are not linear since they contain an exponential; hence we take logarithms to the natural base of both sides of each of them, and obtain equations which are now linear in logarithms. The aforementioned equation appears as follows after logarithms to the natural base have been taken of both its sides:

$$\text{Log } K = \log A + n \log t - ct$$

in which all symbols are as before.

Solving the three linear equations simultaneously and obtaining the three constants A, n, and c in their numerical form for the particular curve, we can then substitute them in the original general equation, and the formula of the special curve in question has been obtained.

In passing it may be mentioned that the three points from which data are taken may be somewhere near the top of the curve, in the region in which baking is likely to be done, since the formula auto-

matically reduces the consistency to zero at zero time and thus actually supplies another point on the curve in addition to the three chosen by the operator. The writer has arbitrarily selected the point of maximum consistency, the point at which the time is half that observed at the maximum consistency, and the point three-quarters of the distance between the time observed at maximum consistency and that observed at the point where the top of the curve crosses the line about which the curve has been centered ("600" line) All of these points are taken on the top of the curve for uniformity although they might just as well be taken in the middle or at the bottom of the curve. These locations are purely arbitrary and are used to provide uniformity of procedure in order that the results of many trials may be reasonably compared. It is possible that further systematic investigations of this process may modify these methods.

The following example will illustrate the procedure which has been outlined. Figure 1 shows a typical farinograph curve selected at

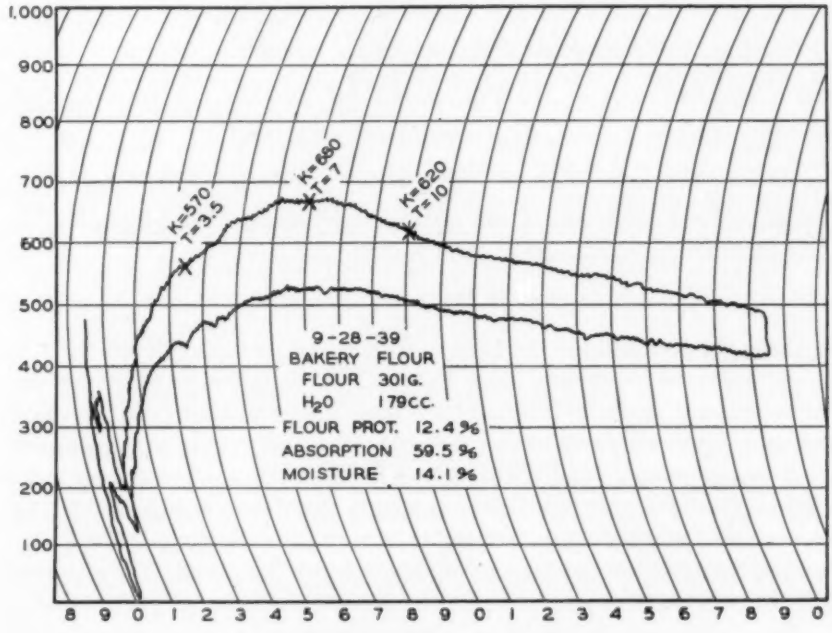

Fig. 1. Formulization of a typical farinogram.

random. It typifies the curve of a bakery flour made by the National Milling Company. Three points are selected, using the procedure above outlined, as indicated by the cross marks on the top of the curve. The coordinates of these points are marked at each point. Substituting

these points in the general equation we have as follows, three equations:

$$570 = A(3.5)^n e^{-3.5c}$$
$$680 = A(7.0)^n e^{-7.0c}$$
$$620 = A(10.0)^n e^{-10.0c}.$$

Taking the logarithms to the natural, or Napierian base, of both sides of these equations we have:

$$\text{Log } 570 = \log A + n \log 3.5 - 3.5c$$
$$\text{Log } 680 = \log A + n \log 7.0 - 7.0c$$
$$\text{Log } 620 = \log A + n \log 10.0 - 10.0c.$$

Looking up these logarithms in tables and substituting their numerical values, we have:

$$6.345 = \log A + 1.253n - 3.5c$$
$$6.522 = \log A + 1.946n - 7.0c$$
$$6.430 = \log A + 2.303n - 10.0c.$$

Solving these equations simultaneously we have:

$\text{Log } A = 5.610$ or $A = 272.0$ (obtained by looking up the number whose log is 5.610)
$n = 1.02$
$c = .153$

Having solved these equations for their unknowns we can now place the unknowns in the original equation and we have:

$$K = 272(t)^{1.0} e^{-.153t},$$

which is the formula for the farinograph curve just considered.

In order to determine how closely this equation fits the actual curve we can make a table of the time and consistency at several different intervals as observed from the original curve, and compare these consistencies thus observed with the values obtained by substituting these same times or intervals in the above formula. If the agreement between these values is close, we can assume that the method has proved satisfactory in this case. The results are given in Table I.

From this table it may be seen that the calculated results were, in all cases, within twenty Brabender Consistency Units of the observed values, indicating an accuracy of about 5%, which is closer than the curve can be read for most purposes. Furthermore the formula is seen to be accurate beyond the last point for which it was calculated, namely t equals ten.

TABLE I

OBSERVED AND CALCULATED CONSISTENCIES

Time (min.)	Consistency (observed)	Consistency (calculated)
0	0	0
2	430	410
4	590	592
6	670	654
8	670	663
10	620	620
12	580	563

The results obtained here are typical of the results obtained in all cases in which the formula has been applied, showing its reliability.

Assuming accuracy and reliability of the formula, the question as to its value and application may be considered.

In this connection two selected lots of flour were mixed in different graduated proportions as indicated in Table II. The farinograph curve for each mixture was obtained and the formulization of each curve was accomplished. The results are given in Table II.

In Table II the whole formula has not been given but only the necessary constants are tabulated.

From an inspection of the results it appears that there is a definite correlation between the mixtures and the arbitrary constants. The figures all proceed one way with few exceptions, and these exceptions may be explained perhaps by the fact that the data are so arbitrarily taken. It has been found that a flour which retains its strength for a long period has low values of its constants and that a high A constant is indicative of a rapid rise of consistency.

The writer can only suggest that correlations may exist between these arbitrary constants and baking or chemical characteristics of the flour.

Precision of Method

From long experience it has been found that the precision is always within 5%, which is possibly greater than the reproducibility of the farinograph curves themselves. This corresponds to an accuracy of about 20 Brabender Consistency Units, or one of the smallest squares on the standard farinograph paper.

Summary and Conclusions

It has been found possible to represent the outlines of farinograph curves accurately by means of a mathematical formula in which there are three arbitrary constants which by adjustment will allow the formula to be fitted with high precision to any farinograph curve.

TABLE II

CALCULATED CURVE CONSTANTS

Flour Lot No. 1		A	n	c
Flour A	Flour B			
%	%			
100	0	487	0.268	0.0396
90	10	451	0.366	0.0468
80	20	379	0.590	0.0765
70	30	512	0.392	0.0653
60	40	485	0.547	0.0915
50	50	566	0.291	0.0549
40	60	576	0.395	0.0936
30	70	653	0.592	0.201
20	80	686	0.739	0.258
10	90	663	0.990	0.382
0	100	906	1.40	0.615
Flour Lot No. 2		A	n	c
Flour C	Flour D			
%	%			
100	0	605	0.145	0.027
90	10	570	0.307	0.0595
80	20	554	0.465	0.0953
70	30	567	0.370	0.0845
60	40	556	0.505	0.1080
50	50	604	0.540	0.1560
40	60	628	0.781	0.261
30	70	742	0.842	0.250
20	80	824	1.08	0.471
10	90	896	0.760	0.402
0	100	940	1.42	0.633

The constants obtained from systematic investigations show variations proportional to the variations of the curves themselves, and probably indicate different characteristics of the flour and wheat. It is suggested that this new method of evaluating the farinograph curves offers a reliable means for characterization, while at the same time incorporating all useful data obtainable from the curves.

It is suggested that the use of this formula for predicting the consistency for any given mixing time would permit the construction of tables by which the miller and baker would have a practical working guide as to the proper mixing time, and would provide as suitable criteria of baking properties as would the curve itself.

It is anticipated that the cereal chemists will find sufficient interest in this method to correlate the arbitrary constants with characteristics of the flour, wheat, or the finished product in order to make the formula useful in predicting as well as in shortening the notation of farinograph curves.

AN APPARENT RELATIONSHIP BETWEEN PROTEIN CONTENT AND RATE OF PROOF[1]

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(Read at the Annual Meeting, May 1940)

There is little doubt that the two main factors involved in the production of bread from flour are (1) enough sugar to produce sufficient gas throughout the entire dough-fermentation period and (2) the ability of the flour dough to retain the gas produced.

It is also true that gas production during the latter stages of fermentation is dependent principally upon the fermentation of maltose produced by diastatic action. Sandstedt and Blish (1934) determined the presence of some substance or substances in flour which have a stimulating effect upon the fermentation of maltose and which they designated as "activators." Blish and Sandstedt (1937) made a study of the properties of this "activator" and determined that it occurs in varying amounts in different flours. They found that malted wheat flours contained significantly higher quantities of this material than did normal flours. They were unable to identify this "activator" but tentatively designated it as "factor M." Sandstedt and Blish (1938) devised a means for estimating the "activator content" of flours by the manometric fermentation technique. A series of 33 flours was observed in baking in an attempt to correlate "activator content" with the time required to proof to a standard height under conditions affording adequate sugar levels in all doughs. Considerable differences in the rate of proof were found, even though the sugar levels were adequate. The conclusion from these results was that in experimental baking investigations proofing should be to a definite height rather than for a constant period of time.

The object of the present paper is to present further evidence (obtained during a study of a number of wheat varieties) which supports the above conclusion.

Methods and Materials

The varietal series of flours used in this investigation were obtained through the courtesy of E. G. Bayfield and the Department of Milling Industry at Kansas State College. They were grown and composited in a manner identical with the series used by Larmour, Working, and Ofelt (1939) in their study of hard winter wheat quality.

[1] Journal Series No. 266, Nebraska Agricultural Experiment Station. Printed with the approval of the Director.

The samples of irrigated Cheyenne and Supreme were obtained from W. O. Whitcomb of the Montana Agricultural Experiment Station. Those of Dawson and Trumbull were obtained from the Federal Soft Wheat Laboratory at Wooster, Ohio. Those of Turkey and Kawvale-Tenmarq were grown in test plots at the Nebraska Agricultural Experimental Station.

The baking formula No. 1 used when proof height was determined was the same as that used by Larmour, Working, and Ofelt (1939) and the procedure the same with the exception that "micro" methods were used. Doughs were based on 25.0 g. of flour (15% moisture basis) and the loaves were baked in pans which were scaled down from the "Markley type" or low form pan. A constant proof time of 55 minutes was used.

The formula used when proof time was determined (No. 2) was as follows: sugar 6%, salt 1%, yeast 3%, shortening 3%, malt flour 0.5%, dry milk solids 6%, and potassium bromate 0.004%. The doughs were proofed to a constant height of 6.1 cm. in pans scaled down from the standard high-form "pup" type pans.

Hydrolyzed gluten, added in certain experiments, was obtained by hydrolyzing 10 g. of ball-milled dried gluten with 1 g. of papain and 1 g. of trypsin for a period of two hours at 30° C. and then boiling for a period of at least twenty minutes to destroy the enzymes.

Experimental

The variety-protein series was baked using formula No. 1. The height of the loaves was measured at the end of the proof period of 55 minutes, at which time the loaves went to the oven. These data are given in Table I. The same series was then baked using formula No. 2 and the loaves were proofed to a standard height of 6.1 cm. The time required to reach this definite height is also recorded in Table I. These data show that when proofing to time there is a definite increase in proof height at increasingly higher protein levels. When proofing to height, the decrease in proof time with increasing protein content is even more striking.

It becomes obvious after an examination of the data in Table I that either the gas retention or the rate of gas production during the proofing period is greater for the high-protein flours than for the lower-protein flours.

The gas-retaining properties of doughs are difficult to determine. Gas production is determined easily and conveniently. Accordingly the latter factor was chosen for study. "Activator content," or third-hour gas production with the flour as the only variable, was determined by

TABLE I

RELATION BETWEEN PROOF HEIGHT AND PROTEIN CONTENT WHEN PROOFING TO TIME AND THE RELATION BETWEEN PROOF TIME AND PROTEIN CONTENT WHEN PROOFING TO A DEFINITE HEIGHT

Per cent protein	Proof height (55 min.)	Proof time (to 6.1 cm.)	Percent protein	Proof height (55 min.)	Proof time (to 6.1 cm.)
	BLACKHULL			CHIEFKAN	
10.0	4.8	47	10.5	4.9	51
11.0	4.8	43	11.1	4.9	51
12.5	5.1	38	12.6	5.1	48
14.3	5.1	35	14.0	5.4	40
15.5	5.3	33	15.1	5.4	39
16.5	5.3	33	16.1	5.5	39
	TENMARQ			TURKEY	
9.8	4.8	47	8.7	4.7	47
10.9	4.8	47	9.4	4.7	46
11.1	4.8	45	10.7	4.9	45
12.3	5.1	43	12.6	5.2	41
13.7	5.4	35	13.7	5.4	37
15.4	5.5	33	15.3	5.4	34
16.9	5.5	29	16.1	5.5	31

Sandstedt-Blish (1938) method on all samples of each of the variety-otein series. The results on four of the varieties are graphically presented in Figure 1. These results show clearly that there is a finite and nearly linear increase in *rate* of gas production during the tical proofing period with increase in protein content. The writers believe that the data in Figure 1 are sufficient evidence to indicate that only present means of eliminating the variable of gas-production rate during the critical proofing period is to proof to a constant height.

Schultz, Atkin, and Frey (1939) have indicated that differences in the third-hour fermentation rate between various flours may be due to differences in their amino-nitrogen content. They determined the effect of 22 amino acids on the third-hour fermentation rate of a patent flour using a yeast-flour-water-maltose mixture. The greatest increase in third-hour gassing rate was produced by the addition of *l*-asparagine. It was found in the course of the present investigation that the maximum effect could be obtained with 25 mg. of asparagine per 10 g. of flour. An attempt was made to bring three flours to a common level of third-hour gas production by the addition of asparagine. The results are given in Table II. It is evident that the addition of asparagine did not bring all flours to a common level of third-hour gas production.

Addition of ball-milled gluten had no effect upon the rate of gas production but the addition of the same amount of this gluten after

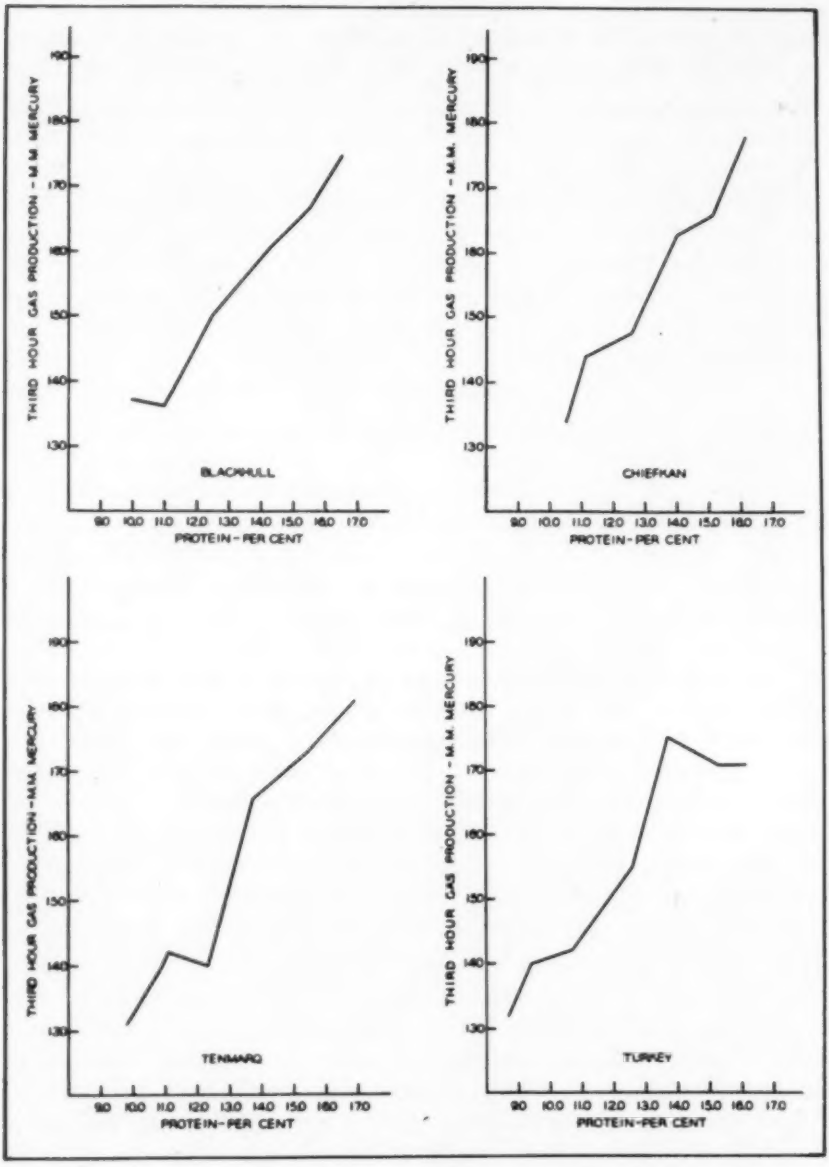

Fig. 1. The relationship between protein content and rate of third-hour gas production.

hydrolysis with papain and trypsin had a very definite effect on the rate of gas production. It was determined that the maximum effect could be obtained by the addition of 300 mg. of the hydrolyzed gluten as shown in Table III. The data in Table IV show that flours obtained

from different varieties of wheat and of widely differing protein contents can be brought to a nearly uniform level of gas production during the critical third hour of fermentation or the proofing period.

Several flours were baked with and without the addition of hydrolyzed gluten in an effort to determine the practicability of the procedure in

TABLE II

EFFECT OF ADDITION OF *l*-ASPARAGINE ON THIRD-HOUR GAS PRODUCTION

Flour	Asparagine added (mg.)	Third-hour gas production (mm. Hg)
Nebr. No. 1	0	157
No. 1	25	185
No. 20	0	143
No. 20	25	193
No. 21	0	153
No. 21	25	176

TABLE III

EFFECTS OF VARYING AMOUNTS OF HYDROLYZED GLUTEN ON THIRD-HOUR GAS PRODUCTION

Flour	Hydrolyzed gluten added (mg.)	Third-hour gas production (mm. Hg)
S. W. Composite	0	174
S. W. Composite	50	203
S. W. Composite	100	223
S. W. Composite	200	228
S. W. Composite	300	231
S. W. Composite	400	233
S. W. Composite	500	233
No. 20	0	140
No. 20	50	178
No. 20	100	204
No. 20	200	207
No. 20	300	212
No. 20	500	209

establishing a common proof time for all flours, but the hydrolyzed gluten had too great an effect upon the dough properties to allow for its practical application.

Since ammonium salts are also known to affect the rate of fermentation, monoammonium phosphate and ammonium chloride were added to flours of widely differing type and "activator content." Data given in Table V show the effects of the monoammonium phosphate on the

TABLE IV

EFFECT OF HYDROLYZED GLUTEN ON THIRD-HOUR GAS PRODUCTION WHEN ADDED TO FLOURS WHICH ORIGINALLY HAVE A WIDE RANGE IN RATE OF GAS PRODUCTION

Variety	Percent protein	Third-hour gas production (mm. Hg)	Third-hour gas production (mm. Hg) with hydrolyzed gluten added
Blackhull	14.3	161	208
Blackhull	15.5	167	214
Blackhull	16.5	175	211
Early Blackhull	15.6	167	213
Chiefkan	14.0	163	211
Chiefkan	15.1	166	208
Chiefkan	16.1	178	216
Cheyenne	10.1	133	209
Cheyenne	13.7	167	208
Kanred	13.9	166	211
Kanred	16.4	173	208
Iobred	13.7	144	200
Tenmarq	12.3	140	196
Tenmarq	15.4	173	204
Turkey	10.7	142	201
Turkey	16.1	171	208

TABLE V

EFFECT OF 0.5% $NH_4H_2PO_4$ ON THIRD-HOUR GAS PRODUCTION AND PROOF TIME WHEN ADDED TO FLOURS WHICH DIFFER WIDELY IN TYPE AND PROTEIN CONTENT

Flour	Percent protein (15% m.b.)	Normal third-hour gas production (mm. Hg)	Third-hour gas production (mm. Hg) with 0.5% $NH_4H_2PO_4$	Normal proof time	Proof time with 0.5% $NH_4H_2PO_4$
Irrigated Cheyenne	7.2	111	196	68	45
Irrigated Cheyenne	10.1	142	199	49	36
Irrigated Supreme	8.3	126	186	54	41
Irrigated Supreme	9.8	143	193	56	41
Kawvale × Tenmarq	13.3	167	192	36	31
S. D. Turkey	14.8	166	194	36	30
Nebr. Turkey	14.7	169	189	34	32
Dawson	7.9	140	195	57	43
Trumbull	9.0	145	197	53	43

rate of gas production during the third hour and also the effect on proof time when these same flours were baked and proofed to a definite height. The data show that, even with flours differing as widely as these in type and protein content, third-hour gas production can be brought surprisingly near to a uniform rate. It is very evident that it is not possible to bring these flours to a uniform proofing time, although

the spread of proof times was reduced by one-half. Undoubtedly this was because of extreme differences in gas-retention properties of doughs from these flours. The possibility still remains that the differences in gas-retention properties among normal hard wheat flours may not be too great to allow all to be brought to a uniform proofing time by the addition of these ammonium salts.

Summary and Conclusions

A number of flours were baked by two methods and it was established that with increasingly higher protein levels (1) proof height increased when proofing to time and (2) proof time decreased when proofing to height.

The rate of gas production during the third hour was determined on the same samples and was found to increase in almost linear relationship with increasing protein content. Proofing to a definite height is the only present means of decreasing if not eliminating the effects of this variable.

It was established that flours could not be brought to a common level of third-hour gas production by the addition of *l*-asparagine.

Hydrolyzed gluten was added to a number of samples of widely differing protein and "activator" contents and it was shown that this brought all flours to a nearly uniform rate of third-hour gas production. This procedure was, however, found to be impracticable in baking.

Ammonium salts were added to a number of flours and the third-hour gas production rate made nearly uniform. Proof time for these flours was not brought to a common level by this procedure although the range of proof times was reduced by one-half.

Literature Cited

Blish, M. J., and Sandstedt, R. M.
1937 Biocatalytic activators specific for the yeast fermentation of maltose. J. Biol. Chem. **118** (No. 3): 765–780.
Larmour, R. K., Working, E. B., and Ofelt, C. W.
1939 Quality tests on hard red winter wheats. Cereal Chem. **16**: 733–752.
Sandstedt, R. M., and Blish, M. J.
1934 Yeast variability and its control in flour gassing power tests. Cereal Chem. **11**: 368–383.
1938 Maltose fermentation activators as affecting baking. Cereal Chem. **15**: 788–794.
Schultz, A. S., Atkin, L., and Frey, C. N.
1939 Fermentation of maltose in the dough. Cereal Chem. **16**: 648–651.

A VARIETAL STUDY OF THE RELATION BETWEEN PROTEIN QUALITY AND PROTEIN CONTENT[1]

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(Read at the Annual Meeting, May 1940)

Comparison of baking qualities of flours of widely varying protein content has always been exceedingly difficult. The chemist, however, is frequently required to make such comparisons. The agronomist wishes to know the relative baking qualities of wheats grown under widely differing conditions, the effects of soil and climate upon protein quality as well as quantity, and whether the quality of a given variety is constant regardless of the protein quantity. The miller wants to know the relative blending values of wheats of differing protein content. Larmour, Working, and Ofelt (1939) propose as a measure of quality the divergence from expectation based on protein content; *i.e.*, the quality is judged by comparing the loaf volume obtained from the flour in question with a standard regression curve obtained from protein-volume values. This should give the relative quality of flours at any particular protein level; however, the difficulty of comparing the qualities of flours at differing protein levels still remains.

Sandstedt, Jolitz, and Blish (1939) have shown that the gluten and starch which have been separated by washing gluten can be recombined to form a reconstituted dough which has baking properties similar to dough made from the original flour. It would then seem reasonable to suppose that wheat starch could be added to a high-protein flour to produce a flour of any lower protein content which may be desired or conversely that gluten washed from a low-protein flour could be added to the original flour to produce a flour with any desired higher protein content. This might afford a method of comparing the baking qualities directly on a basis of a constant protein level. Such a method might also prove to be a means for determining blending quality.

If one accepts the suggestion that protein content and strength are synonymous (Blish and Sandstedt, 1935; Larmour, Working, and Ofelt, 1939) and that the deviation from the expected potentiality is a measure of quality, it would seem imperative that the maximum loaf volume be obtained in baking. Aitken and Geddes (1934) and Larmour, Working, and Ofelt (1939) have indicated the possibility of obtaining the maximum volume from a single bake using a formula which is optimum or nearly optimum for all flours. If this is possible it simplifies the

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work of determining quality even though it does not simplify the determination of flour characteristics. The responses, tolerances, and requirements of a flour must still be determined by the use of supplementary procedures.

Methods

The formulas proposed as optimum vary considerably and the question naturally arises as to their applicability to all flours. Accordingly, in studying the possibility of testing all flours on a definite protein basis, it was thought best to determine whether the absence or inclusion of some of the commonly used supplementary ingredients might affect the judgment of relative quality. It was decided to use the A. A. C. C. basic formula with 6% sugar with supplements of shortening (3%), malted wheat flour (0.5%), and dry milk solids (6%). The milk used was obtained from the same source as that used by Larmour, Working, and Ofelt (1939). Each of these formulas was further supplemented with 1, 2, and 4 mg.% of potassium bromate.

The micro baking technique, using 25 g. of flour, described by Van Scoyk (1939) was used through this work after Van Scoyk's claims for the method had been verified. All doughs were mixed to the so-called "optimum" in a National micromixer. This point was determined by observing the transition of the mixing dough from the characteristic rough appearance of the undermixed dough to the velvety smoothness of a well developed dough. The doughs were molded by passing through the National dough sheeter set at 5/32 inch and rolling up as lightly as possible by hand.

The following four experimentally milled, unbleached samples of flour were chosen for this investigation because of their differing yet representative characteristics:

Fulcaster, 9.9% protein.
Blackhull, 15.8% protein.
Tenmarq, 14.4% protein.
Nebred, 16.1% protein.

Each flour was baked undiluted and after dilution with starch (prepared by the method described by Sandstedt, Jolitz, and Blish, 1939) to a 10% protein level.

Effect of Formulas on Determination of Quality

The results obtained on the original undiluted flours are shown in Figure 1. It is seen that for the milk-free formulas the maximum volume was obtained with 2 mg.% of bromate. The addition of milk

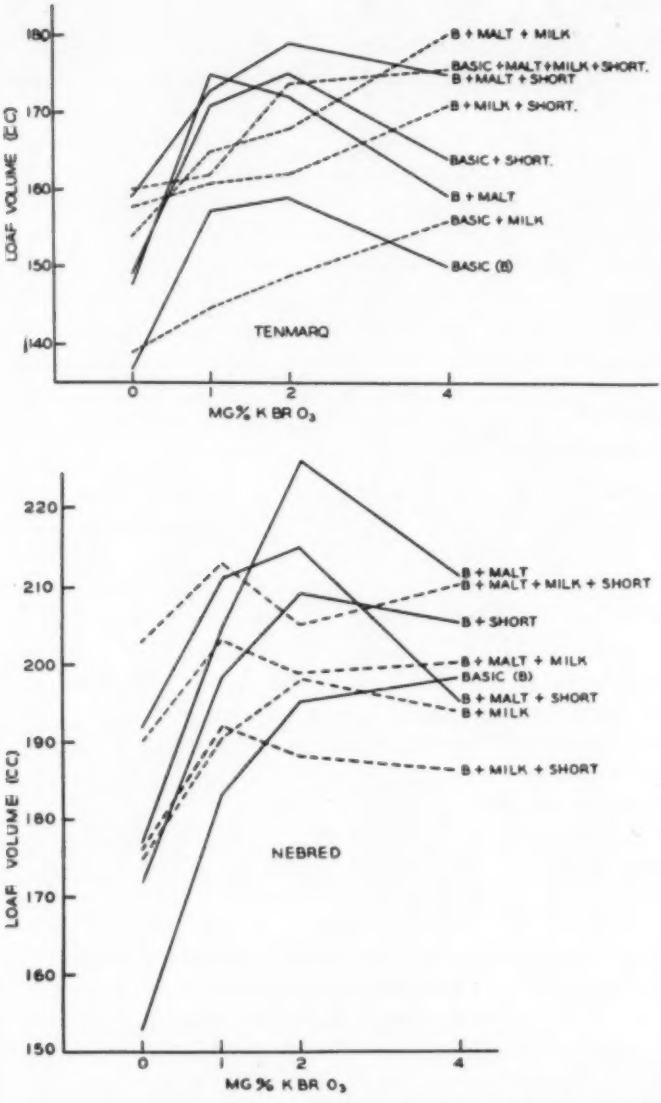
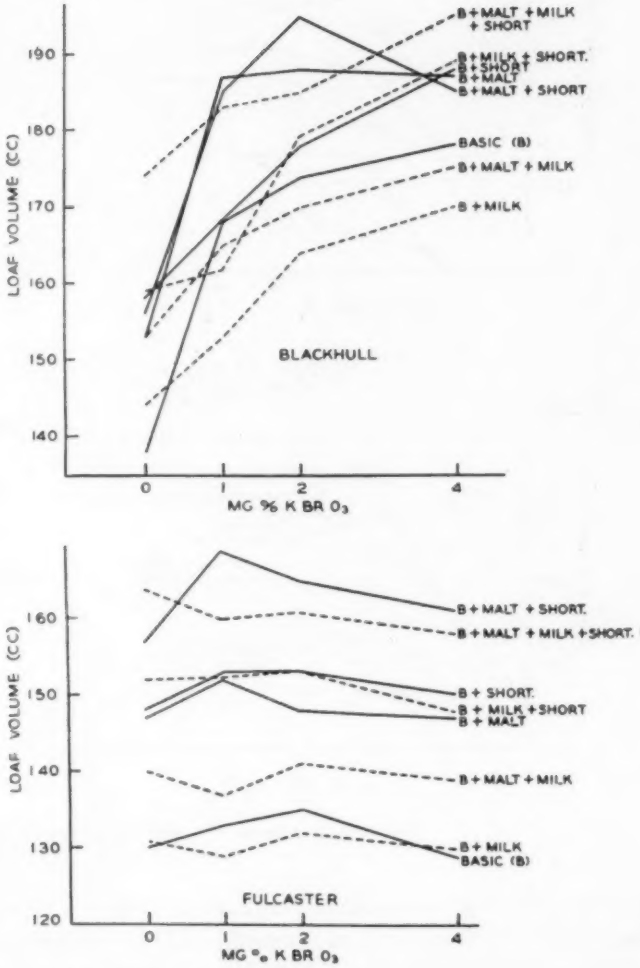


Fig. 1. Comparison of loaf volumes obtained by

to the formulas increased the bromate requirements for the Blackhull and Tenmarq samples to 4 mg.% (or more). Shortening and malt each increased the volume and when both were used the effect was roughly additive. The similarity between curves for the milk-free formulas for each variety indicates that the use of shortening and/or malt does not materially change the bromate requirements, responses, or tolerances.



variations in the baking formula on natural flours.

The results obtained by baking these flours at an artificially produced 10% protein level are shown in comparison to the Fulcaster with a natural 10% protein in Figure 2. The maximum response in the milk-free doughs was obtained with 1 mg.% of bromate; with milk in the formula the requirement was increased to 2 mg.% (or more).

A comparison of the maximum loaf volumes obtained on the four flours is shown in Table I. These results show that addition of shorten-

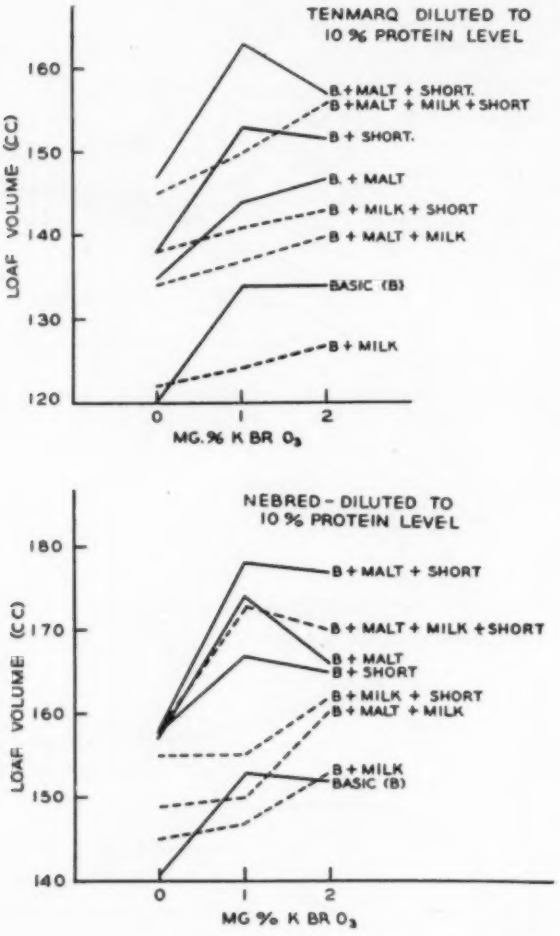
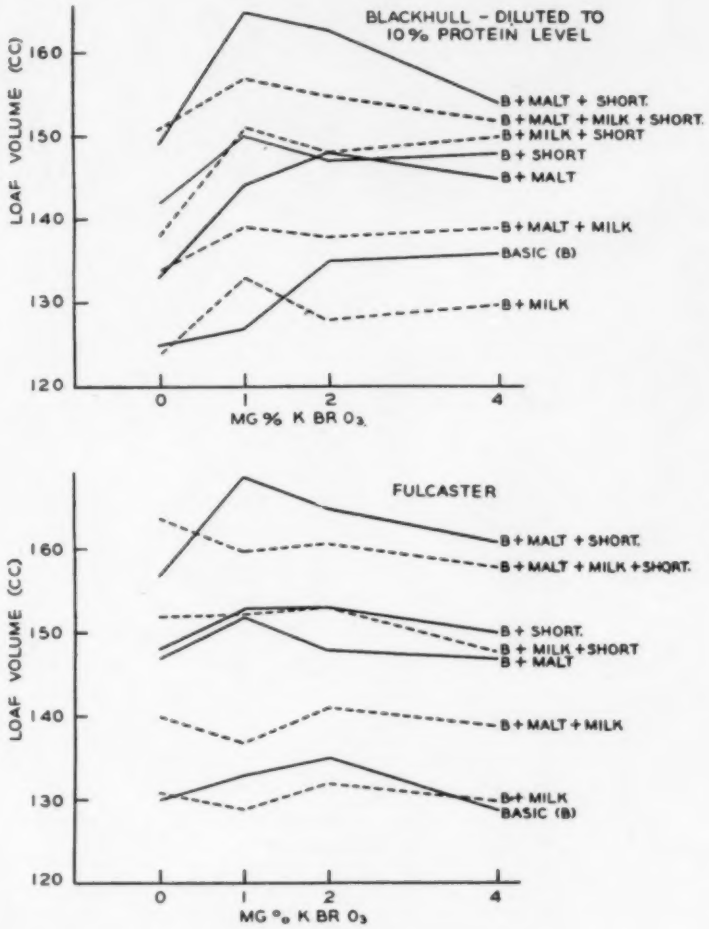


Fig. 2. Comparison of loaf volumes obtained by variations in
(Fulcaster 10%

ing, malt, and milk to the formula has a similar effect on the maximum loaf volume obtained from each of these flours. If the relative value of these flours were to be judged from the loaf volume they would be placed in the same sequence regardless of whether the formula contained malt, milk, or shortening; *i.e.*, the original flours produced loaves which ranged in volume in the order of increasing protein content and the use of supplements did not change this order. This would not necessarily hold true with any other series of flours, since the stimulation obtained by each supplement varies with the flour.

The results obtained by baking the flours at an artificially produced



the baking formula on flours brought to a 10% protein level. original protein.)

10% protein level show that it may be possible to use this method to determine quality. The Tenmarq and Blackhull samples gave practically identical loaf volumes with the original Fulcaster, which had a protein content of 10%, while the Nebred sample gave decidedly higher volumes. It would seem from these data that the inclusion of malt, milk, and shortening is not essential for the determination of quality. We are unable to explain the lack of volume stimulation obtained with milk in these bakes, since this definitely does not agree with the results obtained by Larmour, Working, and Ofelt (1939, 1940) or Ofelt and Larmour (1940).

TABLE I

LOAF VOLUMES OBTAINED AT OPTIMUM BROMATE LEVEL WITH VARIOUS FORMULAS

Formula	Fulcaster 9.9% protein	Tenmarq 14.4% protein	Blackhull 15.8% protein	Nebred 16.1% protein	Tenmarq, diluted to 10% protein	Blackhull, diluted to 10% protein	Nebred, diluted to 10% protein
Basic (B)	135	159	178	198	134	136	153
B—shortening	153	175	188	209	153	150	167
B—malt	152	175	188	226	147	148	174
B—milk	132	156	170	198	127	133	153
B—malt—short.	169	179	195	215	163	165	178
B—malt—milk	141	180	175	203	140	139	160
B—milk—short.	153	171	189	192	143	151	162
B—malt—milk—short.	164	175	195	213	156	157	173

Relation of Quality to Protein Level

The comparative quality of protein at various naturally occurring protein levels may only be determined by studying samples of flour covering a wide range of protein for each of several pure wheat varieties. We were fortunate in obtaining such a variety-protein series of flours through the courtesy of E. G. Bayfield and the Department of Milling Industry of Kansas State College. These samples were baked in duplicate by the same micro technique as described above, using the A. A. C C. basic formula supplemented with dry milk solids (6%), shortening (3%), malted wheat flour (0.5%), and 4 mg.% of potassium bromate. The loaf volumes obtained are shown graphically in Figure 3. Each curve represents a single variety over a range in protein content. It is notable that there was an exceedingly wide spread in loaf volumes between varieties at any give protein level. The difference increased with increasing protein content. This might indicate that there are greater differences in protein quality at the higher levels.

These flours were diluted to a 10% protein level and rebaked. The formula used differed from that used on the undiluted flours only in that it contained no milk and the bromate was reduced to 1 mg.%. The loaf volumes obtained were plotted against the original protein content of the samples and are illustrated in Figure 4. There was a marked difference between varieties in loaf volumes obtained. The curves for all varieties have a strong tendency to be similar in shape, the shape indicating that the highest-quality protein in any one variety was found in flour which had a naturally occurring protein content of 12% to 13%; perhaps in this particular series the climatic conditions which produced this protein level happen to be the conditions favorable for the production of high baking quality. The protein decreased in quality as the

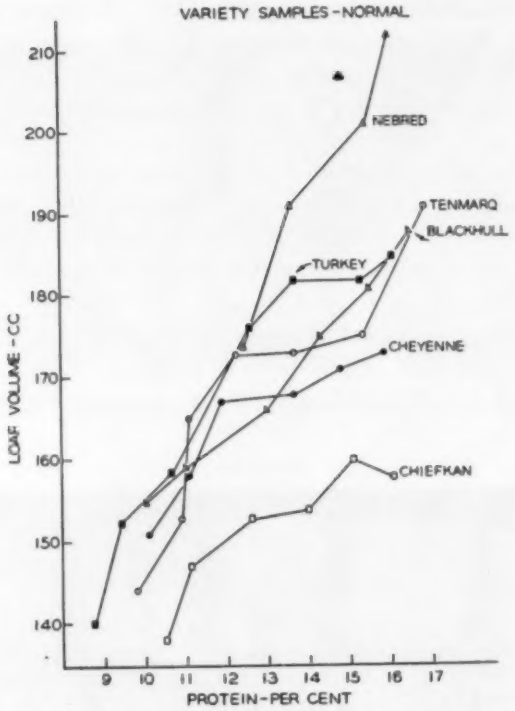


Fig. 3. Loaf volumes obtained on the original variety samples.

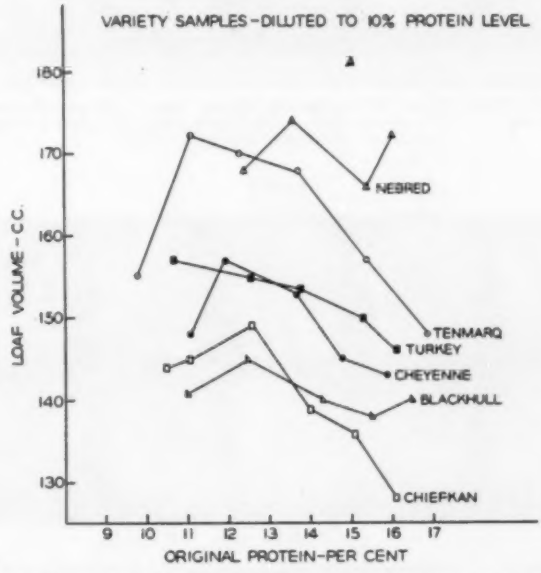


Fig. 4. Loaf volumes obtained on the variety samples reduced to a 10% protein level.

percentage increased above approximately 13%. It should be noted that the high-protein Blackhull flour grown at Lincoln, Nebraska, which was used in the preliminary survey of methods (Figs. 1 and 2), had high protein quality, suggesting that the poor quality indicated in some of the samples may be environmental rather than varietal. Further evidence of this probable effect of environment is given in Figures 8 and 9 by the isolated triangles representing a 15% protein Nebred sample grown at Lincoln, Nebraska. It should be noted that these curves indicate ratings for the varieties that differ from those indicated by the original undiluted flours (Fig. 3).

Figures 5, 6, 7, 8, 9, and 10 show the external appearance of the loaves baked from this variety-protein series of flours. The upper loaves represent the original flours; the lower loaves represent in each

Tenmarq

11.1 12.3 13.7 15.4 16.9

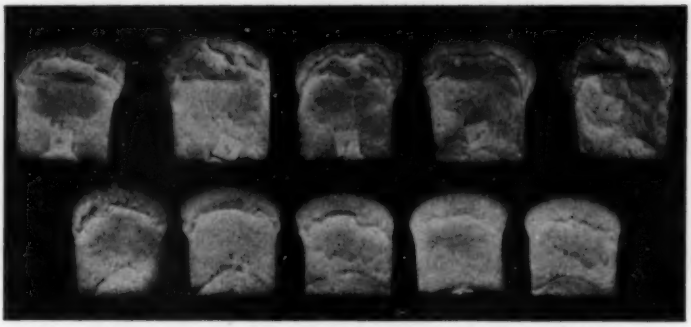
Fig. 5. Comparison of loaves baked from the original Tenmarq flours with loaves baked from the same flours diluted to a 10% protein level. The figures indicate original protein content of the flour.

Turkey

10.7 12.6 13.7 15.3 16.1

Fig. 6. Comparison of loaves baked from the original Turkey flours with loaves baked from the same flours at an artificial 10% protein level.

Blackhull



11.0 12.5 14.3 15.5 16.5

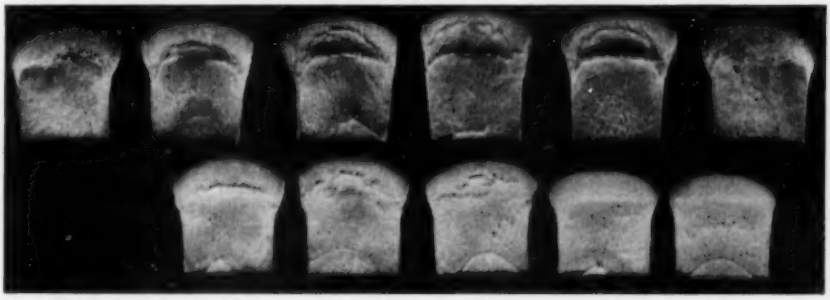
Fig. 7. Comparison of loaves baked from the original Blackhull flours with loaves baked from the same flours at an artificial 10% protein level.

Nebred

12.4 13.6 15.4 16.1

Fig. 8. Comparison as above for Nebred.

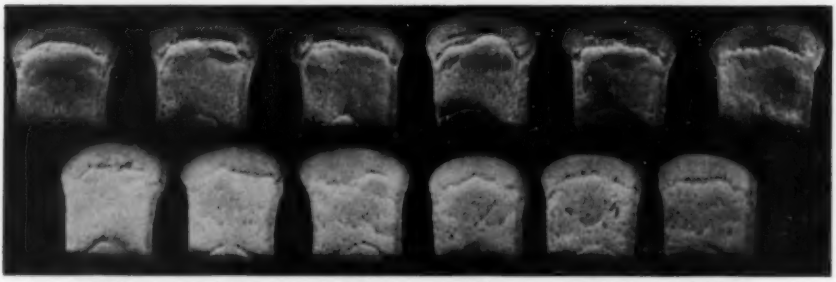
Cheyenne



10.1 11.1 11.9 13.7 14.8 15.9

Fig. 9. Comparison as above for Cheyenne.

Chiefkan



10.5 11.1 12.6 14.0 15.1 16.1

Fig. 10. Comparison as above for Chiefkan.

case the same flour diluted to a 10% protein level. The figure under the loaf indicates the original protein content of the flour. The indication of retrogradation of protein quality with increasing protein content is evident though the variation between varieties in this respect is very marked. Judging from the results obtained on the artificially produced 10% protein flours, Tenmarq (Fig. 5) seemingly had very high protein quality in the intermediate protein range with a rapid decrease with increase in protein content. The quality of the Turkey flours (Fig. 6) was intermediate and quite uniform at all levels. The Blackhull samples (Fig. 7) had rather poor quality over the entire protein range, while the Nebred samples (Fig. 8) had exceedingly high quality even in the high protein ranges.

Summary

A method is suggested for comparing the protein quality of flours at a definite protein level.

The applicability to the study of quality of supplements of malt, shortening, and milk to the A. A. C. C. basic formula was investigated. Van Scoyk's micro-baking technique was used. All supplements gave the same comparative evaluations of the flours when the oxidation and mixing times were optimum for the individual flours. The comparative evaluations of flours reduced to a definite protein level differed from the comparative evaluation of the original flours.

A study was made of the comparative quality of the proteins of wheat varieties at various naturally occurring protein levels. The data indicate that there is wide variation in protein quality between varieties and that the protein quality within a variety varies with the naturally occurring protein level. It is suggested that the results obtained by this protein dilution method may be an indication of the blending value of flours.

Literature Cited

Aitken, T. R., and Geddes, W. F.
1934 The behavior of strong flours of widely varying protein content when subjected to normal and severe baking procedures. Cereal Chem. **11**: 487–504.
Blish, M. J., and Sandstedt, R. M.
1935 Definition and measurement of "flour strength" as an inherent property of wheat. Cereal Chem. **12**: 653–664.
Larmour, R. K., Working, E. B., and Ofelt, C. W.
1939 Quality tests on hard red winter wheats. Cereal Chem. **16**: 733–752.
1940 Quality tests on soft red winter wheats of Kansas. Cereal Chem. **17**: 18–29.
Ofelt, C. W., and Larmour, R. K.
1940 The effect of milk on the bromate requirements of flours. Cereal Chem. **17**: 1–18.
Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.
1939 Starch in relation to some baking properties of flour. Cereal Chem. **16**: 780–792.
Van Scoyk, W. V.
1939 Micro-baking technique application and results. Cereal Chem. **16**: 1–12.

INCREASE OF VITAMIN B_1 INTAKE BY THE USE OF SPECIAL HIGH VITAMIN B_1 BREAD[1]

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According to the recent report of the Mixed Committee on the Problem of Nutrition of the League of Nations (1938): "There is strong evidence that human dietaries in many parts of the world are more or less deficient in vitamin B_1 although beri-beri is absent." That inadequacies may be commonly encountered in American dietaries is indicated by the reviews of Williams and Spies (1938) and Jolliffe (1938).

Baker, Wright, and Drummond (1937) have traced the progress of flour refining and have indicated the changes which this progress has made in the vitamin B_1 content of the diet. The amount of thiamin in whole wheat is not greatly in excess of that found in several other foods. However, interest is rightly centered in this substance since it has been shown that over 20% of the caloric intake of the American diet is contributed by wheat. For this reason wheat, if not deprived of its vitamin B_1 content by milling procedures, may contribute very significantly to the total intake of the vitamin. However, according to the values given by the investigators cited above, 100 g. of modern white flour contains

[1] This investigation was aided by a grant from Standard Brands, Inc.
[2] With the technical assistance of Gordon H. Magruder.

only 7% as much vitamin as 100 g. of whole wheat and their surveys indicate that refined white flour accounts for well over 80% of the sales of wheat products for human use.

The need for the addition of vitamin B_1 to staple American foods has recently been stressed by Cowgill (1939). The use of a yeast produced under conditions so that it has a high content of vitamin B_1 has been shown by Schultz, Atkin, and Frey (1939) to restore the thiamin content of white bread so that it is equivalent to whole-wheat bread.

The evaluation of the state of thiamin nutrition by determining the output of the vitamin in the urine has been proposed by Harris and Leong (1936). This has led to the development of several methods for the determination of thiamin (Levine and Marples, 1938). The present report presents studies carried out on seemingly normal young women in which special white bread of high thiamin content was included in the diet in moderate amounts.

Experimental

Seventeen well nourished young women who expressed a willingness to cooperate served as the subjects in this study. For the most part these were students at Western Reserve University. A preliminary control period was observed during which a 24-hour sample of urine was collected. Hemoglobin estimations and red cell counts were also made during this time. At the end of the control period, which varied from one to three weeks, the subjects were supplied with the special bread and instructed to substitute this bread for the bread ordinarily consumed, but to make no other alterations in their ordinary diet. The subjects ate this bread for approximately five weeks. During this time tabulation of the total food intake for seven consecutive days was made, and from these data the vitamin B_1 intake was calculated as well as the proportion of the total intake of vitamin B_1 supplied by the bread. Hemoglobin estimations and red cell counts were made at fortnightly intervals, and at the end of the bread-eating period two such estimations were made on different days. A second 24-hour sample of urine was collected at the end of this period. The subjects also filled out a questionnaire as to any subjective symptomatic changes that might have been observed during the period of eating the high vitamin B_1 bread.

Estimation of hemoglobin was carried out in all cases by the acid hematin method and by the benzidine method of Bing and Baker (1931). Red cell counts were made as described in standard texts on hematology.

The composition of the diets was calculated from the tables of Waller (1937). The preferred values for the thiamin content of foods given

by Daniel and Munsell (1937) and Williams and Spies (1938) were used in calculating the thiamin intakes.

Method for estimation of urinary thiamin.—The urinary thiamin was determined by a modification of the yeast fermentation method of Schultz, Atkin, and Frey (1937, 1938). As suggested by these authors, normal urine appears to contain substances in addition to thiamin which accelerate the rate of yeast fermentation. A technique was used in which the stimulating effect of urine was compared with a similar amount of urine in which the thiamin had been destroyed by treatment with alkaline ferricyanide. Two additional fermentation mixtures were employed which contained urine that had been treated to destroy its original thiamin and to which had been added 2 and 4 gamma of crystalline thiamin.[3] From the stimulatory effect of the known amount of thiamin the concentration of thiamin in the urine was calculated. Schultz and his associates measured the fermentation rate by catching the evolved CO_2 in manometers. We have used this method and have also on the same samples determined the concentration of residual sugar in the fermentation mixtures and thus have evaluated sugar consumption.[4] Values based on calculations of gas production or sugar consumption agree within the limits of the experimental error of the method. It is quite possible of course to carry out analyses for thiamin without the use of manometers if residual sugar estimations are made. These can conveniently be made by saturating a portion of the fermentation mixture with picric acid and then applying the colorimetric sugar method of Myers and Bailey (1916).

Under the conditions employed, the treatment with alkaline ferricyanide always resulted in complete destruction of thiamin if it was added to the urine. Numerous recovery experiments were carried out in which it was possible to ascertain the amount of thiamin that had been added to the urine. In the studies reported below duplicate determinations were carried out on all of the urine specimens.

Source of bread.—The bread used in this study was baked twice weekly by a local bakery. It was a plain white bread baked with a high vitamin B_1 yeast. Assays of the thiamin content of the bread were carried out each week by the fermentation method.

Results

The effect of the ingestion of the high vitamin B_1 bread on the hemoglobin concentration and the red cell counts of the subjects is shown in Table I. Inspection of the individual values indicates that

[3] Kindly supplied by Merck and Co., Inc., Rahway, New Jersey.
[4] This type of procedure was suggested by Dr. J. A. Killian.

TABLE I

BLOOD FINDINGS IN SUBJECTS EATING HIGH VITAMIN B_1 BREAD

Subject	Average during initial control period		Average at end of bread-eating period	
	Hemoglobin	RBC count	Hemoglobin	RBC count
	g. per 100 cc.	*Millions per cu.mm.*	*g. per 100 cc.*	*Millions per cu.mm.*
1	12.3	4.7	12.2	4.4
2	10.7	4.6	9.8	4.3
3	13.0	4.7	12.6	4.5
4	13.0	4.5	13.0	4.6
5	12.7	4.9	13.4	5.2
6	13.1	4.1	11.4	4.2
7	12.7	4.8	13.7	5.0
8	12.5	4.8	12.8	4.8
9	12.5	4.4	12.3	3.9
10	14.6	4.9	13.7	4.9
11	12.0	4.1	12.9	4.6
12	13.4	4.3	13.0	4.6
13	12.4	4.8	12.5	4.9
14	12.7	4.9	11.8	4.5
15	11.9	4.5	12.0	4.3
16	12.6	4.3	12.0	4.6
17	12.5	4.1	12.6	4.3
Average	12.6	4.6	12.5	4.6

the maximum increase in hemoglobin was 1.0 g. per 100 cc. of blood, whereas the maximum decrease was 1.7 g. The average values for the control period and for the analyses at the end of the bread-eating period are practically identical. There were also no significant changes in the red cell counts.

The effect of the ingestion of high vitamin B_1 white bread on the urinary excretion of thiamin is shown in Table II. The individual values for thiamin excretion vary widely, the lowest value being 39 gamma of thiamin per 24-hour sample and the highest 450 gamma per 24-hour sample. Of the 17 subjects, 14 showed an increase in the urinary thiamin excretion after the period of ingestion of the high vitamin B_1 bread. Of the three subjects who showed a decrease in urinary thiamin two were out of school for two or three days with influenza just prior to collection of the second 24-hour urine specimen. The average figures for the group indicate that the urinary thiamin output was nearly doubled as a result of the ingestion of the vitamin B_1 bread.

A summary of the information obtained from the daily diet records of the subjects appears in Table III. The percentage of the total thiamin intake contributed by the bread is seen to be 34.1%, which is a very considerable proportion.

Of the 17 subjects, 15 reported that they liked the bread and two

TABLE II

URINARY THIAMIN EXCRETION

Subject	Thiamin excretion during control period before eating vitamin B_1 bread	Thiamin excretion at end of period of ingestion of vitamin B_1 bread
	Gamma per 24 hours	*Gamma per 24 hours*
1	39	154
2	118	275
3	102	94
4	72	82
5	258	346
6	74	157
7	73	80
8	53	121
9	73	162
10	271	151
11	53	330
12	92	450
13	100	152
14	87	43
15	71	396
16	180	350
17	79	151
Average	106	206

TABLE III

SUMMARY OF DIETARY RECORDS OF SUBJECTS EATING HIGH VITAMIN B_1 BREAD

Subject	Age	Weight	Protein intake[1]	Fat intake[1]	Carbohydrate intake[1]	Caloric intake[1]	Thiamin intake[1]	Percent of thiamin intake contributed by B_1 bread
	yrs.	*kg.*	*g.*	*g.*	*g.*	*cal.*	γ	
1	35	56	64	98	180	1858	913	37.6
2	28	49	97	96	224	2150	1155	30.8
3	20	56	70	78	182	1710	738	39.4
4	25	66	103	164	238	2840	1400	28.2
5	20	57	85	107	219	2179	1134	23.2
6	23	47	62	89	284	2185	911	36.9
7	20	58	70	93	198	1909	1041	37.4
8	20	52	73	87	225	1975	1110	31.5
9	36	70	73	102	249	2206	1251	34.6
10	30	54	78	107	249	2271	1245	37.8
11	22	66	74	74	150	1562	984	32.6
12	24	53	90	115	197	2183	1222	33.3
13	20	60	66	69	231	1807	1005	27.9
14	20	57	59	85	207	1829	933	45.3
15	32	69	77	58	223	1721	1076	37.8
16	33	48	63	86	194	1802	942	34.1
17	22	44	73	87	203	1887	986	31.6
Average	25	57	75	94	215	2004	1062	34.1

[1] Average daily intake.

reported that they did not particularly like any kind of bread but that this was as good as any. The approximate average daily consumption over the whole period was between four and five slices. Four of the subjects reported that they definitely felt better while eating the bread and six reported an increase in appetite. No other changes in subjective symptoms were reported.

Discussion

For some time there has been a vague suggestion of relationship between vitamin B_1 and secondary anemia. This is indicated by the policy of some physicians to include vitamin B_1 in the treatment of certain secondary anemias. It is also suggested by the policy of some of the pharmaceutical houses who recommend preparations for anemia which are fortified with vitamn B_1.

Minot (1939) recently stated that more information was needed regarding the relationship between chronic iron deficiency and some factor of the vitamin B complex. Summerfeldt and Ross (1938) found that the addition of vitamin B_1 and iron to a "good" diet of normal children produced increases in hemoglobin concentration of the blood and increases above the expected growth rate. Schlutz, Oldham, and Morse (1938) have suggested that increases in vitamin B_1 intake result in a decrease in iron retention in the normal infant.

The exact status of our subjects was somewhat questionable. In light of present-day hematological standards, the hemoglobin concentrations were definitely below the figure that is normally given for such an age group. However, for three successive years surveys in this laboratory of the hemoglobin levels in similar groups have given almost identical results. Furthermore if this is a true anemia it does not respond to administration of iron salts since a large group of such students did not show hemoglobin increases when fairly large doses of iron were given (unpublished observations from this laboratory). In the present study the ingestion of increased amounts of vitamin B_1 as supplied by the bread did not appear to have any effect on the hemoglobin concentration or the red cell counts in these subjects. If a true mild anemia existed it must have been due to other factors.

Since the original work of Harris and Leong (1936) numerous studies of thiamin excretion have been made. Jolliffe, Goodhart, Gennis, and Cline (1939) noted that definite clinical symptoms of deficiency developed in four of five male subjects when the excretion level dropped below 100 gamma per 24 hours. Melnick, Field, and Robinson (1939) have found a definite difference in the excretion levels of male and female subjects. These investigators found that a group of nine women

consuming their ordinary diet excreted 93 gamma of thiamin per 24 hours. The calculated dietary intake of thiamin was 710 gamma per 24 hours. In the subjects of the present study the daily thiamin excretion during the control period was 106 gamma and from the dietary records it might be assumed that they receive approximately 750 gamma of thiamin per day. The agreement between these two series is quite striking. Melnick, Field, and Robinson suggest that a thiamin excretion of 60 to 70 gamma per 24 hours for an adult female represents a borderline condition with respect to thiamin intake. In their series and our own the excretion was not greatly above this level nor was the amount of thiamin in the diet greatly in excess of the minimum requirement suggested by Cowgill (1938). In the series of Melnick, Field, and Robinson (1939) the women were wives of hospital staff members and in the present series the subjects were young college women. In our series we feel that in no case was the ordinary diet greatly restricted by the economic status of the subject.

With the inclusion of the vitamin B_1 bread in the ordinary diet a very definite increase in the average thiamin excretion in the urine was noted at the end of the bread eating period. Approximately 20% of the thiamin intake appeared in the urine. This increase in percentage of excretion might be expected since the increased vitamin intake continued for a period of 5 weeks during which time there was an opportunity for any depleted reserves to be restored. Furthermore the possibility of partial destruction of thiamin in the gastro-intestinal tract which has been suggested as occurring when large doses are given during the fasting state (Melnick *et al.* 1939) is minimized since the extra thiamin was obtained from the food eaten with the three daily meals. There can be little question that if thiamin excretion reflects the level of thiamin nutrition (Melnick *et al.*, 1939; Jolliffe *et al.*, 1939) the subjects in this study were in a much better state of thiamin nutrition as a result of substituting the special vitamin B_1 white bread in place of that ordinarily included in the diet.

It is realized that analysis of diet records is subject to a considerable error as far as the amounts of foods are concerned. Furthermore a marked variation occurs in the composition of different samples of the same food. The dietaries of this group of subjects during ingestion of the special bread as indicated by Table III included adequate amounts of vitamin B_1. The last column of Table III indicates the amount of thiamin contributed by the high vitamin B_1 bread, and it is to be seen that the thiamin content of the bread makes up over 34% of the total thiamin intake. In these diets the bread contributed approximately 16% of the caloric intake. It is well recognized that low-income groups have the most limited thiamin intake. In these same diets bread contributes

a larger percentage of the total caloric intake. In such low-income groups the use of vitamin B_1 bread might reasonably be expected to cause more marked improvement in the thiamin intake than noted in the present study.

In regard to the subjective symptoms very little can be said since this merely represents the reports of the subjects. However the possibility that the changes in general well being and in appetite reported by 25% of the subjects were real rather than psychological cannot be denied.

Summary

White bread baked with a new high vitamin B_1 yeast was ingested by 17 young college women in place of the bread ordinarily included in the diet. The somewhat subnormal blood hemoglobin concentrations of these subjects did not show any alteration during this period. The urinary thiamin excretion before the period of eating the special bread averaged 106 gamma per 24 hours, whereas at the end of the five-week period the excretion of thiamin averaged 206 gamma per 24 hours. If the urinary excretion reflects the state of thiamin nutrition, this indicates an improved state of thiamin nutrition in these subjects. Dietary records obtained from the subjects indicated that the special bread contributed 34% of the total thiamin intake.

Literature Cited

Baker, A. Z., Wright, M. D., and Drummond, J. C.
1937 The nutritive value of bread. J. Soc. Chem. Ind. **56**: 191–194.

Bing, F. C., and Baker, R. W.
1931 The determination of hemoglobin in minute amounts of blood by Wu's method. J. Biol. Chem. **92**: 589–600.

Cowgill, G. R.
1938 Human requirements for vitamin B_1. J. Am. Med. Assoc. **111**: 1009–1016.
1939 The need for the addition of vitamin B_1 to staple American foods. J. Am. Med. Assoc. **113**: 2146–2151.

Daniel, E. P., and Munsell, H. E.
1937 Vitamin content of foods. U. S. Dept. Agri. Misc. Pub. No. 275.

Harris, L. J., and Leong, P. C.
1936 Excretion of vitamin B_1 in human urine and its dependence on dietary intake. Lancet **1**: 886–894.

Jolliffe, N.
1938 A clinical evaluation of the adequacy of vitamin B_1 in the American diet. New International Clinics **IV**, Series **48**: 47–67.

——, Goodhart, R., Gennis, J., and Cline, J. K.
1939 The experimental production of vitamin B_1 deficiency in normal subjects. The dependence of the urinary excretion of thiamin on the dietary intake of vitamin B_1. Am. J. Med. Sci. **196**: 198–211.

League of Nations
1937 Final report of the mixed committee of the League of Nations on the relation of nutrition to health, agriculture, and economic policy. Columbia University Press, New York. No. **A13**, p. 62.

Levine, S. Z., and Marples, E.
1938 The status of the color tests for vitamin B_1. Nutrition: The newer diagnostic methods. Milbank Memorial Fund, New York. 91–101.

Melnick, D., Field, H., and Robinson, W. D.
1939 A quantitative chemical study of the urinary excretion of thiamin by normal individuals. J. Nutrition **18**: 593–610.
Minot, G. R.
1939 Anemias of nutritional deficiency. A symposium on the blood and blood-forming organs. University of Wisconsin Press, Madison, Wisconsin.
Myers, V. C., and Bailey, C. V.
1916 The Lewis and Benedict method for the estimation of blood sugar with some observations obtained in disease. J. Biol. Chem. **24**: 147–161.
Schlutz, F. W., Oldham, H., and Morse, M.
1938 Effect of vitamin B_1 on the iron retention of a normal infant. Am. J. Dis. Child. **56**: 735–743.
Schultz, A. S., Atkin, L., and Frey, C. N.
1937 A fermentation test for vitamin B_1. J. Am. Chem. Soc. **59**: 2457–2460.
1938 The specificity of the fermentation test for vitamin B_1. J. Am. Chem. Soc. **60**: 3084–3085.
1939 The vitamin B_1 contents of wheat, flour and bread. Cereal Chem. **16**: 643–648.
Summerfeldt, P., and Ross, J. R.
1938 Value of increased supply of vitamin B_1 and iron in the diet of children. Am. J. Dis. Child. **56**: 985–988.
Waller, D. S.
1937 Nutritive value of foods. George Wahr, Publisher, Ann Arbor, Mich.
Williams, R. R., and Spies, T. D.
1938 Vitamin B_1 (thiamin) and its use in medicine. The Macmillan Company, New York.

THE THIAMIN CONTENT OF WHOLE-WHEAT AND CLEAR FLOURS

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(Received for publication May 13, 1940)

The fermentation method for the determination of thiamin as developed by Schultz, Atkin, and Frey (1937, 1938) has given the baking technologist a useful tool for the study of the vitamin B_1 content of his raw materials. Compared to feeding tests, the fermentation method is rapid and inexpensive, and therefore of great value in a study of this kind. The data presented in this report (Tables I–IV) are taken from continuous routine assays, and many of the tests were made on flours actually used in production. Before a complete picture of the vitamin B_1 content of flours used in this country can be drawn, statistical studies from many sources as well as different methods of assay must be evaluated, and for this reason these tests should be of interest and serve a useful purpose.

Williams and Spies (1938) have summarized the literature and report variations of from 4.7 to 10.2 gamma of thiamin per gram in whole-wheat flours, and with the preferred average from 4 to 5 gamma

TABLE I

THIAMIN CONTENT OF SPECIAL WHOLE-WHEAT FLOURS

(Data calculated to the 10% moisture basis)

Source	Ash	Thiamin	Source	Ash	Thiamin
	%	*Gamma/gram*		%	*Gamma/gram*
New York State	1.74	6.8	Pacific Coast	1.56	7.0
NewYork State	1.48	5.2	Durum (N.W.)	1.69	6.6
New York State	1.78	7.0	Kansas	1.71	7.0
Oklahoma	1.66	6.6	Kansas	1.88	7.6
Texas	1.60	7.7	Kansas (all Chiefkan)	1.71	7.2
Texas	1.75	8.0	Kansas (all Tenmarq)	1.51	5.0
Virginia	1.77	6.7	Kansas (allTurkey)	1.82	6.7
Maryland	1.79	5.2	Northwestern	1.79	7.9
Ohio	1.83	6.4	Northwestern	1.65	8.0
Pacific Coast	1.61	7.8	Northwestern	1.81	6.5
Pacific Coast	1.53	7.5	Southwestern	1.80	7.8
Pacific Coast	1.35	6.0	Southwestern	1.46	5.9

Average: 6.8 gamma per gram

TABLE II

THIAMIN CONTENT OF COMMERCIAL SHIPMENTS OF WHOLE-WHEAT FLOUR

(Data calculated to 10% moisture basis)

Ash	Thiamin	Ash	Thiamin
%	*Gamma/gram*	%	*Gamma/gram*
1.75	5.3	1.58	6.6
1.75	8.7	1.54	6.9
1.81	7.9	1.77	6.5
1.78	7.6	1.76	6.7
1.77	8.2	1.51	6.5
1.81	7.1	1.57	6.6
1.76	6.7	1.55	6.2
1.84	8.0	1.62	6.6
1.56	6.7	1.74	6.7
1.77	7.8	2.05	6.4
1.55	7.7	1.58	5.7

Average: 6.9 gamma per gram

TABLE III

VARIATION OF THIAMIN CONTENT OF WHOLE-WHEAT BREAD WITH THE THIAMIN CONTENT OF THE FLOUR USED

Flour (10% moisture basis)		Bread (38% moisture)	
Gamma/gram	*Int. units/lb.*	*Int. units/lb.*	*Decrease, %*
5.3	800	460	42.5
7.8	1182	650	45.0
6.5	984	550	44.0

TABLE IV

THIAMIN CONTENT OF CLEAR FLOURS

(Data calculated to the 13% moisture basis)

Ash	Thiamin
%	*Gamma/gram*
0.534	2.7
0.540	3.4
0.563	2.8
0.574	2.0
0.615	2.3
0.684	3.1
0.698	2.6
0.706	3.0
0.710	2.5
0.710	3.1
0.712	2.8
0.723	2.6
0.727	4.3
0.753	4.4
0.754	3.9
0.760	3.6
0.764	4.1
0.808	4.7

per gram. These figures are taken from feeding tests and from the bradycardia method of assay, and a considerable number of them represent the thiamin content of flours available in England.

The decrease in vitamin B_1 content from the flour to the bread represents a combination of factors: first, the dilution due to the higher moisture content of the bread as compared to the flour; secondly, the addition of nonvitamin-containing ingredients such as shortening, sugar, and salt; and thirdly, a slight baking loss.

Copping and Roscoe (1937) summarized the literature then available on clear flours. The results reported indicated that the lower the grade of the flour, the higher the vitamin content. Schultz, Atkin, and Frey (1939) found clear flours to contain 2.7 and 1.8 gamma of thiamin per gram, and a second-grade clear to contain 5.1 gamma per gram.

In Figure 1 some values for cake, standard-patent, and straight-patent flours were included in order to illustrate further the variation of the thiamin content with the ash of the flour. It is interesting to note that whatever the degree of extraction or the source of the flour, the thiamin content bears a quite definite relationship to the ash.

Summary

As determined by the fermentation method the average thiamin content of 46 samples of whole-wheat flour was 6.85 gamma per gram on

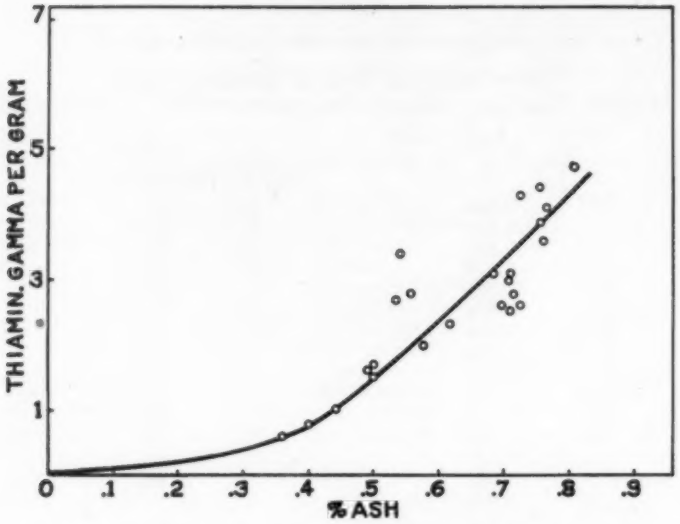

Fig. 1. The relationship between thiamin content and the ash of flour.

the 10% moisture basis. There was no correlation with ash content and no special trend toward high or low vitamin contents in various parts of the wheat-producing areas except that the white or softer type of wheat tended to be somewhat lower than the average. Most of the samples tested were no doubt blends of wheat varieties and hence no correlation with varieties could be suggested.

The thiamin content of clear flours had a definite relationship to the ash content and varied from 2.0 to 4.7 gamma per gram.

Literature Cited

Copping, A. M., and Roscoe, M. H.
1937 The water-soluble B-vitamins in yeast, flour and bread. Biochem. J. **31**: 1879–1902.
Schultz, A. S., Atkin, L., and Frey, C. N.
1937 A fermentation test for vitamin B_1. II. J. Am. Chem. Soc. **59**: 2457–2460.
1938 Influence of nicotinic acid on the fermentation method for vitamin B_1. J. Am. Chem. Soc. **60**: 1514.
1939 The vitamin B_1 content of wheat, flour and bread. Cereal Chem. **16**: 643–648.
Williams, R. R., and Spies, T. D.
1938 Vitamin B_1 and its use in medicine. The Macmillan Co.

THE LOSS OF THIAMIN IN BREAD ON BAKING AND TOASTING

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The feeding tests which have been made on bread and bread ingredients in order to obtain the vitamin B_1 content indicate that the baking loss is small. For example, Copping and Roscoe (1937) pointed out that the similarity of the vitamin contents of the breads and the flours from which they were made suggested no appreciable destruction of the vitamin during baking. Williams and Spies (1938) reported that the vitamin content of the bread on the air-dried basis was similar to that of the flour from which the bread was made. Aughey and Daniel (1940) found that there was a baking loss of 15% in whole-wheat bread. These results suggest that the baking loss is probably of a degree which approaches the limit of accuracy of a feeding test.

The fermentation test (Schultz, Atkin, and Frey, 1937, 1938) gives results which indicate that the baking losses vary from 5% to 9% of the total vitamin originally present. Objections may be made to the use of the fermentation test in this type of study because of the possible yeast-stimulating power of break-down products of the thiamin. If such were entirely true, it would be difficult to show any baking loss by this test. Any such possible error, as will be seen, cannot exceed the allowable error of duplicate feeding tests, and the fermention test therefore gives a satisfactory method for studying the variation in thiamin content of the different portions of the slice, a study which would be difficult with feeding tests.

In order to determine the thiamin content of different portions of the slice, and thereby measure the baking loss, slices were divided as follows: (1) the caramelized part of the crust, (2) one-half inch of the slice immediately under the crust, and (3) the interior of the slice (one square inch). These various portions were air-dried, ground, and the thiamin determined. The results in all cases were calculated to the dry basis.

The assumption was made that no destruction of thiamin took place in the immediate center of the loaf, and therefore the baking loss was taken as the percentage difference between the thiamin content of the interior of the slice and the average of the entire slice. The average baking loss of the three types of bread shown in Table I was 7%.

No doubt the end slices would lose proportionally more than the

TABLE I

THIAMIN CONTENTS OF VARIOUS PORTIONS OF THE SLICE

(Thiamin in gamma per gram of bread calculated to the dry basis)

Type of bread	Crust	Next to crust	Interior	Entire slice	Baking loss
Regular white	1.0	1.2	1.2	1.1	8%
High B_1 white	6.9	8.7	10.8	10.2	5%
Whole wheat	5.3	6.7	7.5	6.8	9%

other slices of the loaf because of the greater surface exposed to the heat of the oven, but figured on the entire loaf, the thiamin content of a slice gives a reliable value for the thiamin content of the entire loaf. During the preparation of a number of loaves for feeding tests, the fermentation test indicated no measurable difference in thiamin content between an average slice and the entire loaf, and apparently any effect of the end slices is lost because of the small percentage of the entire loaf represnted by the end slices.

Since a rather large portion of the bread consumed in this country is in the form of toast, the stability of thiamin during toasting is of nutritional importance. In order to study the loss of vitamin during toasting, a number of slices from the same loaf were taken and light, medium, and heavy toast prepared. It was difficult of course to obtain exactly the same degree of toasting for the different breads studied, but it is believed, however, that the heavy toast represented considerably more toasting than the average person would prefer. After toasting, the slices, including an untoasted slice serving as a control, were air-dried, ground, and tested for vitamin B_1 by the fermentation method. Results are shown in Table II, and for Melba toast in Table III.

TABLE II

LOSS OF THIAMIN ON TOASTING

(Thiamin in gamma per gram calculated to the dry basis)

Type of bread	Untoasted	Light toast	Medium toast	Heavy toast	Percentage loss		
					Light	Medium	Heavy
Regular white	1.0	1.0	1.0	1.0	0%	0%	0%
High B_1 white	4.9	4.7	4.2	3.7	4%	10%	24%
High B_1 white	12.3	10.8	10.2	9.7	12%	17%	21%
Whole wheat	6.7	6.7	6.5	5.9	0%	3%	12%

TABLE III

THE LOSS OF THIAMIN IN THE PREPARATION OF MELBA TOAST

(Thiamin in gamma per gram calculated to the dry basis)

Type of bread	Untoasted	Melba toast	Percentage loss
Regular white	1.1	1.0	9%
High B_1 white	10.2	7.5	26%

Summary

The baking losses of thiamin in three types of bread vary from 5% to 9% as measured by the fermentation test.

The toasting losses of thiamin (medium toasting) vary from 0% to 17%, depending on the type of bread. The process of making Melba toast is more destructive of thiamin than ordinary toasting.

Literature Cited

Aughey, E., and Daniel, E. P.
1940 Effect of cooking on the thiamine content of foods. J. Nutrition **19**: 285–296.

Copping, A. M., and Roscoe, M. H.
1937 The water-soluble B-vitamins in yeast flour and bread. Biochem. J. **31**: 1879–1902.

Schultz, A. S., Atkin, L., and Frey, C. N.
1937 A fermentation test for vitamin B_1. II. J. Am. Chem. Soc. **59**: 2457–2460.
1938 Influence of nicotinic acid on the fermentation method for vitamin B_1. J. Am. Chem. Soc. **60**: 1514.

Williams, R. R., and Spies, T. D.
1938 Vitamin B_1 and its use in medicine. The Macmillan Co.

SOME PHYSICAL AND CHEMICAL CHANGES OCCURRING IN HARD RED SPRING WHEAT GLUTEN DURING DOUGH FERMENTATION

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The effects of dough fermentation upon the properties of wheat gluten have received substantial attention in cereal chemistry literature. Since gluten forms the framework of the dough, holding in its interstices the other substances such as starch, sugar, and yeast cells which are present, any changes induced in the properties of gluten during fermentation are of direct practical importance.

Proteolysis is probably one of the most important factors concerned in fermentation. Too great a proteolytic activity will cause the dough to become distinctly slack, and may even render it entirely unfit for bread-making purposes. Too little activity, on the other hand, may cause a dough to be harsh and "bucky." The hydrogen-ion concentration of the reaction medium has a profound influence upon enzymatic activity and protein behavior. The effects of fermentation and of proteolytic enzymes upon dough and gluten characteristics have been extensively studied, with emphasis upon viscosity changes as indicative of proteolytic effects.

Henderson, Fenn and Cohn (1919) believed that a dough with low viscosity would produce a better loaf of bread than one with high viscosity, and thought that the principal advantage in bringing a dough to a pH of 5 before baking was the minimum viscosity obtained at that hydrogen-ion concentration. Sharp and Gortner (1924) postulated that viscosity of a water suspension prepared from a dough, with electrolytes removed, increased to a maximum as fermentation progressed. In a similar way the viscosity of such a suspension treated with lactic acid markedly increased as fermentation proceeded. Malt extract greatly decreased these effects, presumably as a result of its proteolytic action upon the gluten. It therefore appears from this work that proteolysis decreased viscosity, as would be expected, but that fermentation with the production of acid had an opposite effect. Olsen and Bailey (1925) did not agree with the findings of Sharp and Gortner, but thought that the increased hydrogen-ion concentration of the flour suspension undergoing fermentation reduced viscosity. These workers did not find much proteolysis caused by yeast in doughs.

Sharp and Elmer (1924) reported that the proteolytic enzymes present in wheat flour were capable of attacking and digesting flour protein significantly during a digestion period of two to three weeks. The method of estimating protein changes was by autolyzing flour suspensions at 35° C. and then determining the proteins soluble in K_2SO_4, ethyl alcohol, etc.

Bailey and Johnson (1924) estimated the loss of carbon dioxide from fermenting doughs by two methods, one involving measurements of expansion of the dough plus loss of CO_2, and the other the expansion of the dough alone. The loss was found to increase rapidly after a lapse of 100 to 180 minutes, and they judged this point to be an indication of the optimum fermentation period. Sharp and Schreiner (1926) used the Sharp and Elmer method of protein analysis after autolysis but did not find marked proteolysis in dough fermentation. The plasticity constants represented by the consistency and the yield value of glutenin

suspensions after treatment with lactic acid did increase, however, as yeast fermentation progressed.

Brownlee and Bailey (1930) also investigated the proteolysis of bread doughs. Determinations of nonprotein nitrogen by protein precipitation with stannous chloride indicated a relatively unimportant cleavage of gluten protein as fermentation progressed, even in dough to which malt preparations had been added. There was no evidence to show that proteolytic enzymes and the by-products of fermentation, such as acid, alcohol, etc., effected gluten degradation during the time range of usual bread-dough fermentation. Viscosimetric technique, however, did show evidence of changes taking place in fermenting doughs, probably due to differences in imbibitional properties of the flour gluten.

Smith (1925) found that flours of high viscosity were of excellent baking quality, while flours of low viscosity ranged from excellent to poor in baking quality. Smith accordingly postulated that in the majority of instances viscosity was not a measure of baking quality. The viscosities were determined upon leached and unleached flour-water suspensions with a Sheely pipette.

Bohn and Bailey (1936) constructed a "stress meter" and measured the stress-strain relationships of fermenting doughs. A significant reduction in the stress readings occurred during fermentation, which suggested to the authors that progressive fermentation "mellows" rather than "develops" wheat-flour dough. It was also found that high stress readings were a good indication of the ability of a dough to withstand prolonged mixing. Later (1937) these workers found that small amounts of papain did not affect stress readings. Larger additions, however, significantly decreased stress readings. They explained these effects by the hypothesis that small increments of the enzyme act as a flour coagulant, whereas larger amounts are liquefying.

The action of various inhibitory substances upon proteases has been extensively discussed in the literature and will therefore not be reviewed in the present paper. The work of Read and Haas, Jorgensen, Flohil, Harris and Johnson (see "Literatured Cited") has called attention to this phase of cereal investigations, with adequate literature references. These researches (especially in the instance of Harris and Johnson) have mainly dealt with the effect of oxidizing agents, such as potassium bromate, upon proteases either native to the flour or added in the form of yeast, papain, pepsin, etc. Harris and Johnson (1940) studied the effects of these enzymes and $KBrO_3$ upon viscosity and other properties of spring wheat gluten dispersions. It was found that papain and pepsin have similar effects upon the rate at which glutens dispersed in 10% sodium salicylate, and that bromate altered this rate. The quantity of protein precipitated from the gluten dispersions by $MgSO_4$ was mark-

edly reduced by papain, but not by pepsin. The mode of procedure in this study was to add the enzyme and bromate in appropriate quantities to the other dough ingredients, mix for three minutes in the Hobart-Swanson to thoroughly incorporate the ingredients in the dough, and immediately wash the gluten from the dough with a standardized stream of 0.1% sodium phosphate solution of pH 6.8. No time was allowed for enzymic action other than during the mixing period.

In view of the results obtained when the substrate was exposed to the action of the enzyme for a maximum of less than five minutes, and with the work of other investigators upon fermenting doughs in mind, it seemed desirable to extend the study to a determination of enzymic effects upon these properties of wheat gluten during normal dough fermentation. The techniques already described were accordingly applied to doughs which were fermented for varying periods of time.

Experimental Material and Methods

A commercially milled hard red spring wheat straight flour was used for this investigation. This flour was not bleached nor had any diastatic agent been added. The protein content of the flour was 13.2% and the ash 0.44% on a 13.5% moisture basis.

The doughs from which the glutens were washed were made up in the manner normally used in this laboratory, using the standard basic formula with 5% of sucrose added to furnish sufficient fermentable material for the yeast during the course of the fermentation. It was decided to ferment the doughs over a period of five hours to cover thoroughly the range of customary experimental baking, and to take samples for washing gluten at hourly intervals starting with the first sample immediately after mixing, as had been done in the former study. Each dough would thus furnish six samples of gluten for investigation, and the effect of fermentation upon gluten properties as related to formula could be followed. The doughs were fermented at 30° C. and 85% relative humidity. Punches were made at hourly intervals.

In addition to using the viscosity and fractionation procedures outlined by the authors in previous publications, determinations of hydrogen-ion concentration and oxidation-reduction potentials were made upon the doughs at hourly intervals, using a Beckmann pH meter with glass and platinum electrodes. Because a test of the physical properties of the gluten as influenced by fermentation seemed desirable, a series of resistance tests were made upon the freshly washed gluten by means of a "tenderness" tester. This apparatus was fully described by Binnington, Johannson, and Geddes (1939) and used by them as well as by Harris and Knowles (1940) in determining the tenderness of cooked

macaroni. The possibility of using the tenderness test to study the resistance of doughs and glutens to pressure was privately suggested by Binnington and Geddes inasmuch as the equipment commonly used for determining gluten qualities depends upon stretching and pulling effects. On the other hand the experimental baker largely forms his opinion of gluten quality during the fermentation period by the "feel" of the dough between his hands when punching and working the dough and the results yielded by the apparatus described should correspond in some degree at least to the baker's judgment. A plunger 50 mm. in diameter was used in these experiments.

A modification was introduced in the method of dispersing the pieces of gluten washed from the doughs in sodium salicylate. In former instances this dispersion had been accomplished by shaking the dispersion flasks by hand, at approximately five-minute intervals, care being taken to prevent foaming. No doubt variations in the degree of shaking would occur between operators and between different shaking periods. It was also impossible to follow the shaking schedule throughout the 24 hours, and this factor no doubt introduced small errors in the final results. A further undesirable source of variation was that possible surface denaturation might occur if the shaking were done too vigorously. In view of these considerations a mechanical rotary shaker was constructed of aluminum mounted upon a wooden base. This equipment, a picture of which is shown in Figure 1, is capable of holding 20 Erlenmeyer flasks of 200-cc. capacity at one time. It has a speed of approximately 14 rpm. and is actuated by a ¼ hp. electric motor. This machine functioned very satisfactorily, no trace of foaming being observed in the dispersions during the process of disintegration, while the rate of revolution was sufficient to insure thorough stirring of the flask contents. A further factor in favor of machine shaking was that there would be little opportunity for mechanical disintegration to affect the gluten dispersion rate because of the gentle agitation of the flask contents.

It was decided to study the effect of additions of 0.002% and 0.004% papain to the standard basic formula. An addition of 0.001% $KBrO_3$ was also made to the standard basic formula, and increments of 0.001% and 0.004% $KBrO_3$ were superimposed upon the 0.004% papain-treated doughs.

Discussion of Results

As the data are conveniently suited to graphic presentations, no tables of results are presented in this paper, and the discussion will be confined to the figures. The comparative rates of dispersion, as registered in centipoises $\times 10^2$ and determined at intervals of four hours, will be discussed first.

Examining the data obtained with the standard basic method, as graphically presented in Figure 2, it is evident that the length of time the dough was fermented had a decided effect upon the viscosity-time relationships. The initial effect of fermentation appeared to slightly increase the rate of dispersion in sodium salicylate, while further fermentation decreased it. This effect reached a maximum after three hours. The difference between the second and third hours is very striking, and may be connected with coagulation of the protein by the proteolytic enzymes present in the dough. After the third hour the rate of dispersion increases again, rising approximately half of the distance to the zero hour curve. This rise is seemingly related to further liquefying action of the proteases as well as to an increase in hydrogen ion concentration in the fermenting dough.

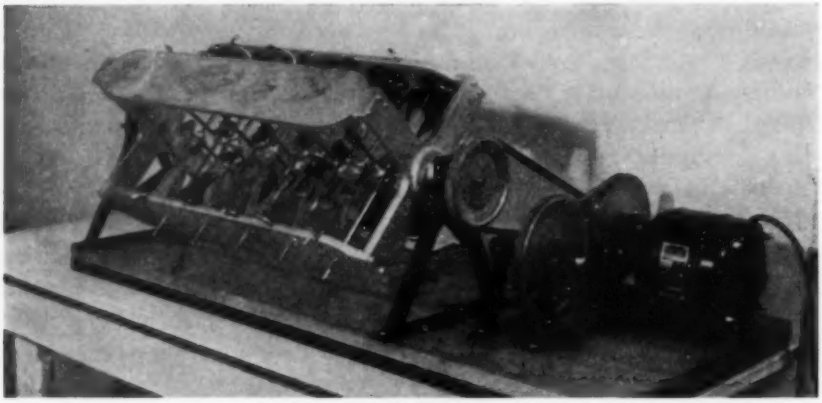

Fig. 1. Motor-actuated rotary shaker used for gently agitating gluten dispersions in 10% sodium salicylate solution during the period of dispersion. Speed-reducing pulleys shown at right. Frame holding flasks is constructed from aluminum and is mounted on steel supports. Rods for fastening flasks in shaker are of brass and are held in correct position by brass thumb screws which can be seen projecting from the sides of the flask carriers. The flasks (200-cc. Erlenmeyer in the photograph) rest in a suitable hollowed-out seat lined with felt. Capacity of shaker, 20 flasks. Speed of shaker, 14 rpm.

Turning next to Figure 3, which represents the data obtained in a similar way with the standard basic formula plus 0.001% $KBrO_3$, the effects of fermentation noticeable in Figure 2 have been increased by the bromate. The zero and first-hour dough curves are very similar to those in Figure 2, but the following four doughs yielded glutens having practically the same dispersion rate. This rate shows a very marked change after the eighth hour of dispersion and probably represents the combined effects of an initial coagulative action by the protease and an inhibitory effect of the bromate, the latter substance acting to prevent further liquefying activity by the proteases.

In Figure 4 the results obtained when 0.002% papain was added to the dough mix are shown. There are no apparent significant differences among the curves representing the glutens washed from doughs fermented for different periods. In comparing this set of results with the preceding figures it is evident at once that the glutens all dispersed

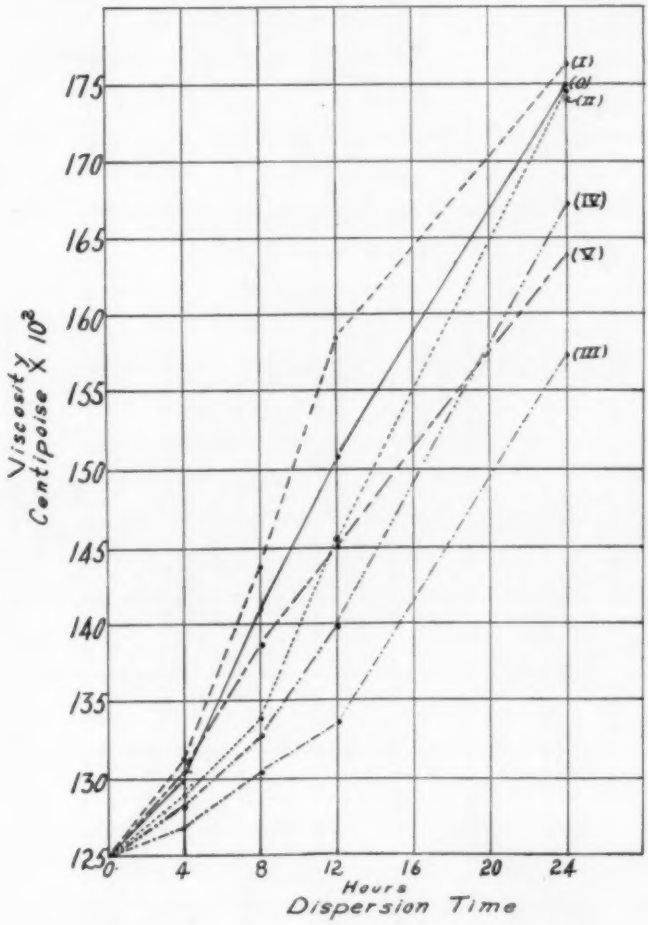

Fig. 2. Relationships between viscosity and time of dispersion of gluten washed from doughs prepared from standard basic formula. Number of curve represents hours of fermentation of dough before gluten was washed.

much more rapidly when treated with papain, and the full effect of the enzyme is apparently felt during the mixing period. There appears to be some evidence in favor of the theory that increasing the fermentation period may have tended to reduce the size of the gluten protein micelle.

The same situation is represented in Figure 5, which shows the relationships when 0.004% papain was added to the dough.

The effect of 0.001% $KBrO_3$ upon 0.004% papain in the dough is

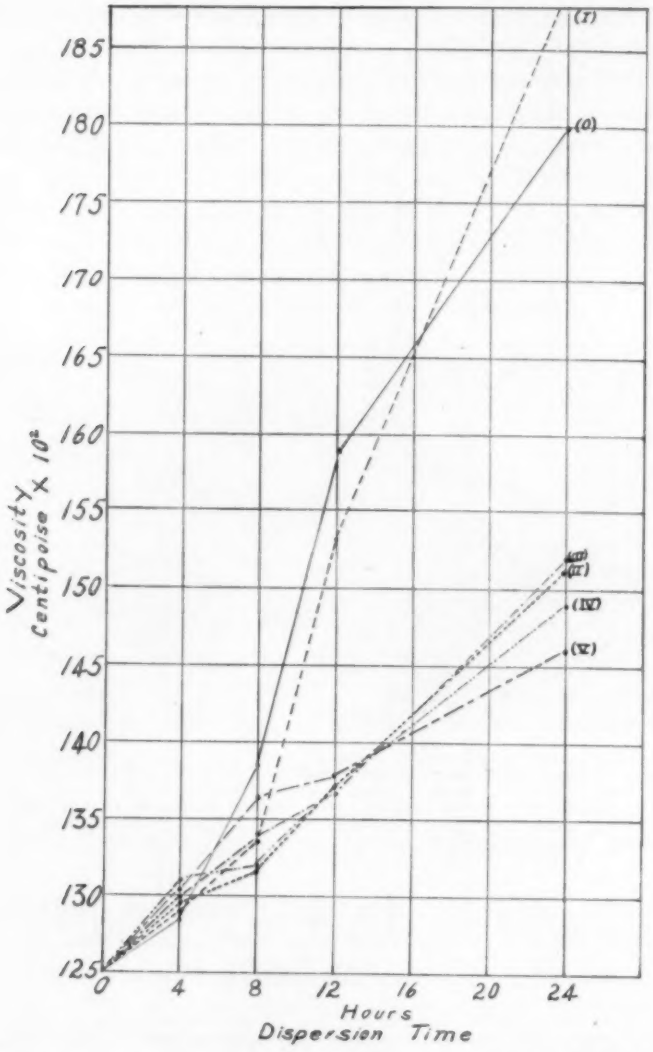

Fig. 3. Relationships between viscosity and time of dispersion of glutens washed from doughs prepared from standard basic formula plus 0.001% $KBrO_3$. Number of curve represents hours of fermentation of dough before gluten was washed.

shown in Figure 6. A gradual decrease in rate of dispersion is evident, this decrease becoming more marked as fermentation time lengthens. No great differences are evident in the unfermented and one-hour-

fermented dough glutens in comparing Figures 5 and 6, thus showing that the bromate did not commence to take effect until the second hour of fermentation was reached. In Figure 7, however, the increase in bromate concentration had a noticeable influence commencing with the

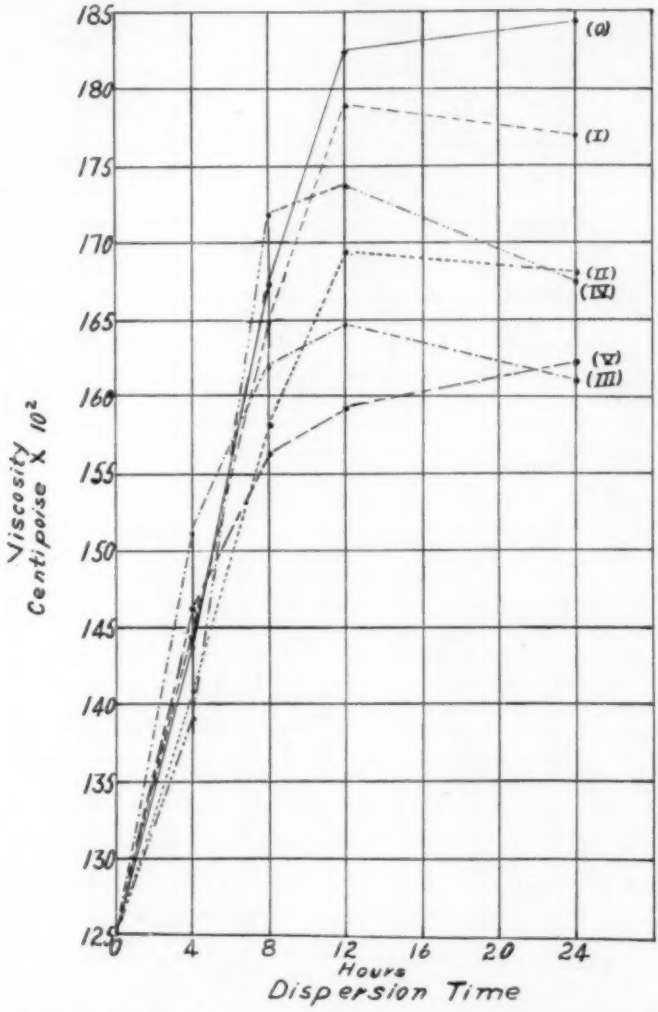

Fig. 4. Relationships between viscosity and time of dispersion of glutens washed from doughs prepared from standard basic formula plus 0.002% papain. Number of curve represents hours of fermentation of dough before gluten was washed.

first hour of fermentation. It is probable that a higher dosage of papain would cause marked differences among the rates of dispersion of the glutens from unfermented doughs, corresponding with results obtained

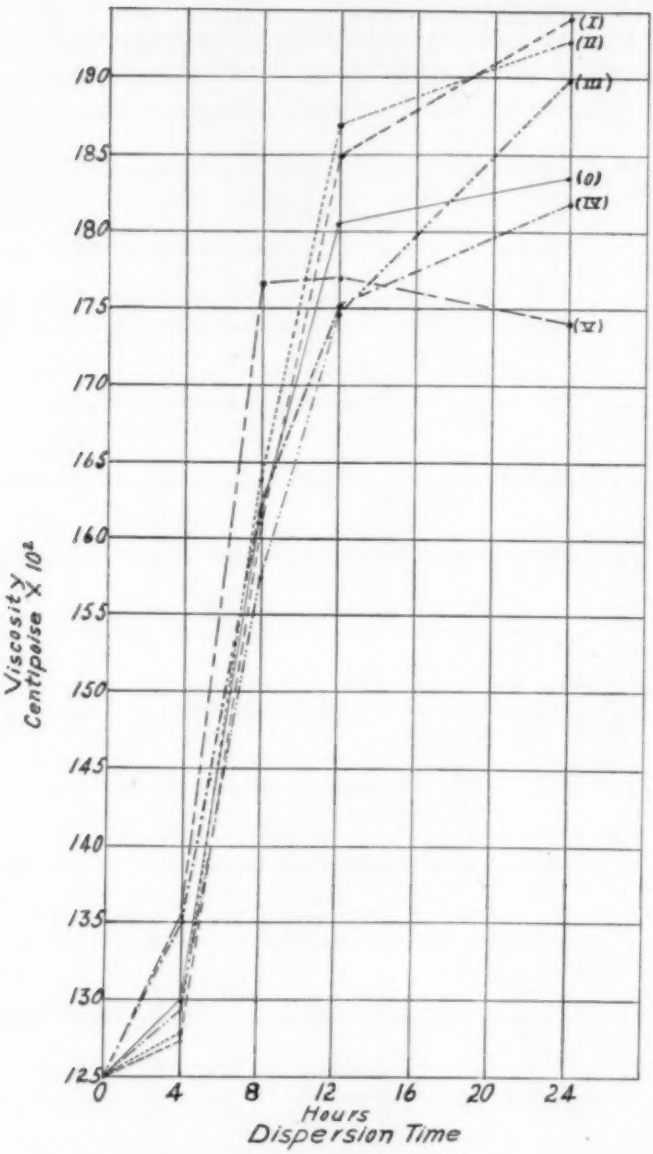

Fig. 5. Relationships between viscosity and time of dispersion of glutens washed from doughs prepared from standard basic formula plus 0.004% papain. Number of curve represents hours of fermentation of dough before gluten was washed.

by the authors in former work of this nature (Harris and Johnson, 1940). It was noticed that the mechanical shaker which was employed in the present study altered the shape of the curves to some extent as

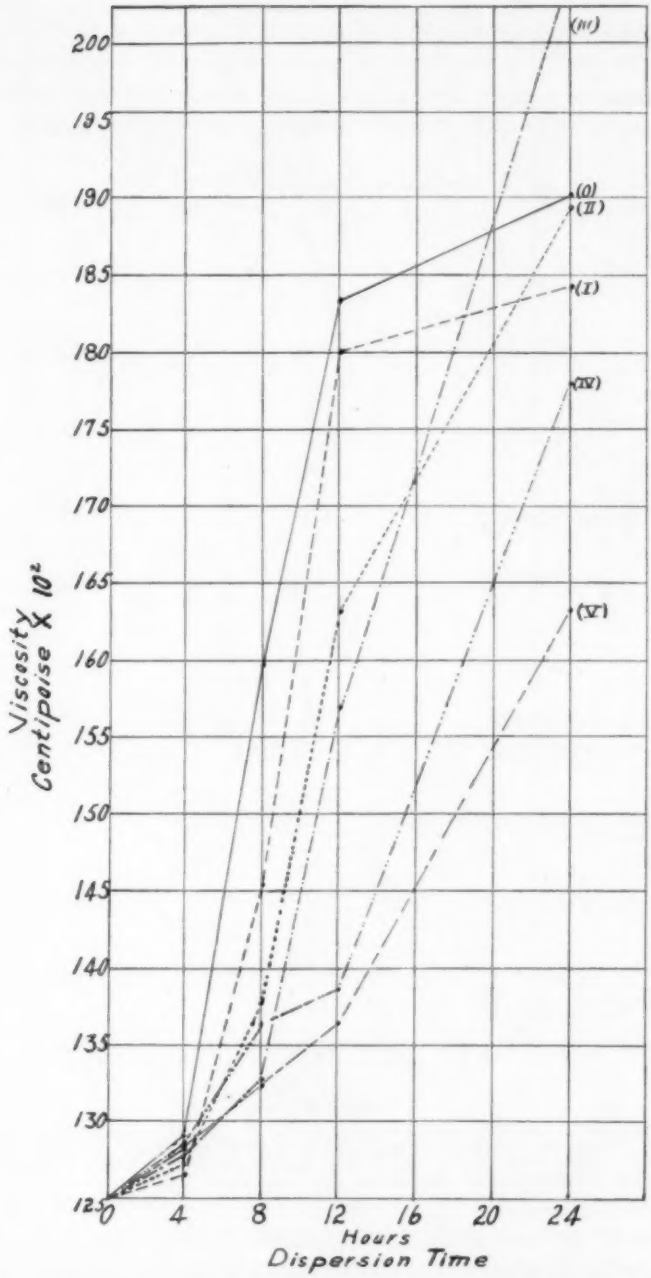

Fig. 6. Relationships between viscosity and time of dispersion of glutens washed from doughs prepared from standard basic formula plus 0.004% papain and 0.001% $KBrO_3$. Number of curve represents hours of fermentation of dough before gluten was washed.

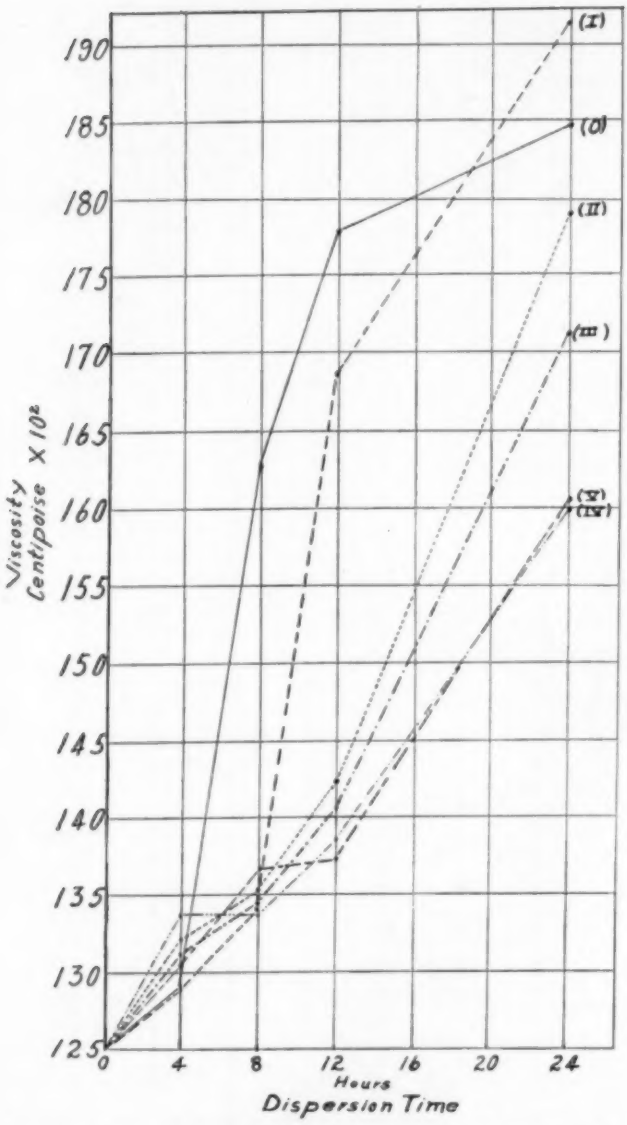

Fig. 7. Relationships between viscosity and time of dispersion of glutens washed from doughs prepared from standard basic formula plus 0.004% papain and 0.004% $KBrO_3$. Number of curve represents hours of fermentation of dough before gluten was washed.

compared to the curves obtained by hand shaking. It is also evident that in the instances of the gluten prepared from the standard basic and bromate-treated doughs which had been fermented for several hours, there was an initial period of " lag " or relatively slow dispersion lasting

from 8 to 12 hours, after which the course of the dispersion was greatly accelerated. This phenomenon could be followed by visual examination of the dispersions. During the first few hours the liquid appeared quite transparent, with the gluten remaining in well defined clumps. After 8 to 12 hours the dispersion began to cloud up and the clumps disinte-

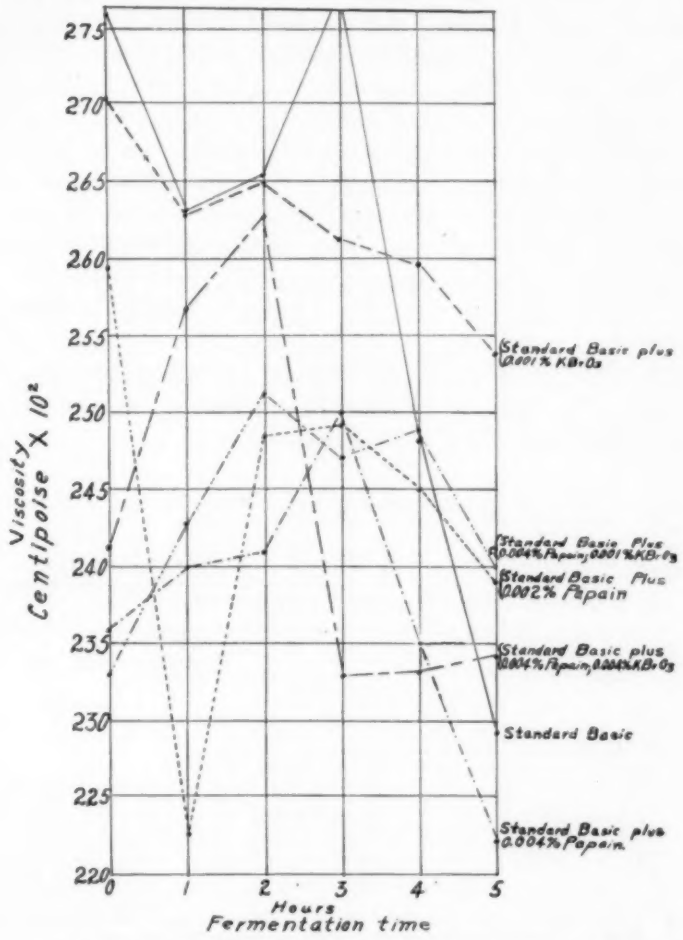

Fig. 8. Relationships between viscosity of gluten dispersion and hours of fermentation for each of the six dough formulas employed.

grated into smaller fragments. In the case of the standard basic-plus-bromate and papain-plus-0.004%-bromate doughs, the effect of the improver was so marked following three or more hours of fermentation that the gluten refused to thoroughly disintegrate after 24 hours of constant shaking.

A further series of dispersions was prepared and continuously agitated on the shaker for 36 hours. They were then centrifuged, and the protein concentration of the liquid was ascertained. The dispersions were next adjusted to a protein concentration of 2500 mg. per 100 cc. and the viscosities determined. In each case the quantity of protein thrown down by the addition of 0.6 cc. of concentrated $MgSO_4$ solution

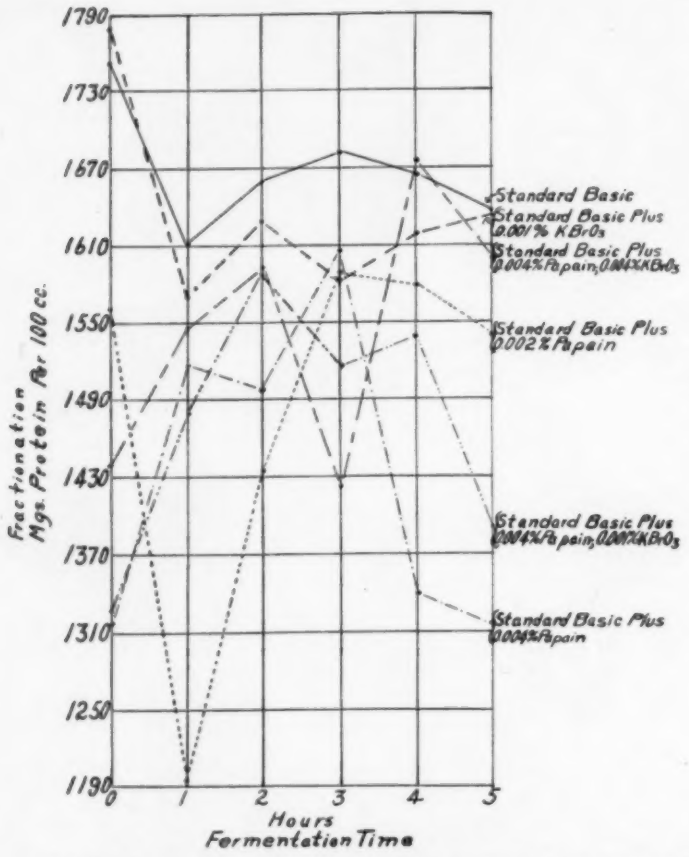

Fig. 9. Relationships between quantity of gluten protein fractionated from dispersion and hours of fermentation time for each of the six dough formulas employed.

to a 10-cc. aliquot of the dispersion was also found. The results obtained, expressed in viscosity and fractionation values for each formula over the fermentation period employed, are shown in Figures 8 and 9, respectively. While a certain amount of variability is evident certain trends can be discerned. From Figure 8 it is evident that in the absence of bromate there is a tendency for viscosity first to decrease and then

to increase to a peak at the second or third hour, followed by a decrease to the end of the fermentation time. In the presence of bromate the initial decrease is modified or disappears entirely and while, in the standard basic doughs, bromate reduces the viscosity peak, it apparently raises this peak in the papain-treated doughs, possibly due to an inhibitory action upon the enzyme. The relationships between fractionation and fermentation time depicted in Figure 9 are roughly similar in nature to the viscosity changes with fermentation period.

Two different effects upon the gluten properties are measured by the studies on the two series of dispersions described. In the first series the influence of the various ingredients and fermentation periods upon the rate of protein dispersion is studied. In the second series the dispersions are on a constant-protein basis, and the effects of formula and fermentation-time variables upon the protein micelle are indicated. These methods of approach to the problem both demonstrate the effect of papain in increasing dispersion rate and reducing effective particle size. Potassium bromate retarded the rate of gluten dispersion and prevented, in some instances, complete dispersion of the gluten. Length of fermentation period apparently increased the effect of bromate upon rate of dispersion, and also affected the size of the protein particle itself.

The changes in pH with fermentation are shown in Table I. A

TABLE I

COMPARATIVE HYDROGEN-ION CHANGES IN FERMENTING DOUGHS

Dough formula	Hours of fermentation					
	0	1	2	3	4	5
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
St. B.	5.85	5.52	5.47	5.37	5.29	5.20
St. B. + 0.001% $KBrO_3$	5.85	5.51	5.46	5.37	5.32	5.27
St. B. + 0.002% papain	5.80	5.48	5.46	5.41	5.32	5.28
St. B. + 0.004% papain	5.83	5.49	5.45	5.38	5.34	5.17
St. B. + 0.004% papain with 0.001% $KBrO_3$	5.79	5.57	5.45	5.40	5.35	5.22
St. B. + 0.004% papain with 0.004% $KBrO_3$	5.79	5.52	5.47	5.35	5.29	5.27

Note—St. B. refers to the standard basic formula with 5% sucrose.

general trend toward a decrease in pH with increase of fermentation time is evident. No differences in pH among the different dough treatments are apparent. These measurements were made directly upon the fermenting doughs by the use of a Beckmann pH meter with the calomel and glass electrodes carefully enclosed in the dough. Each

value shown is the average of five consistent readings. The oxidation-reduction potential values were erratic, and showed no definite trends. Decreasing pH at constant E_h is commonly thought to denote a tendency toward a decrease in oxidizing power.

A third series of glutens was used for the determination of resistance to pressure with a tenderness tester. In using this apparatus the method of Binnington, Johansson, and Geddes (1939) was followed. The glutens were washed from the doughs and the tests run with minimum loss of time. The gluten was formed into a ball and located under the plunger of the apparatus. Five tests were made on each sample,

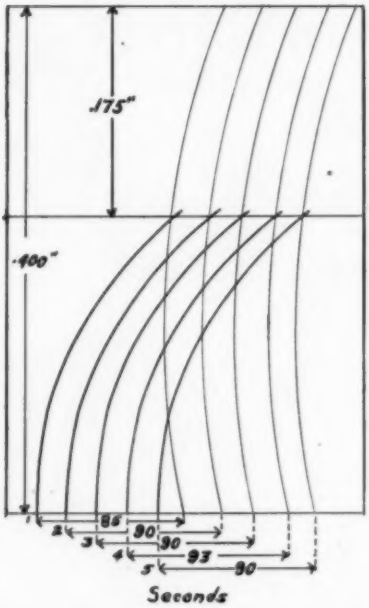

Fig. 10. Representative curves made by five successive determinations of resistance on one sample of gluten. The greater the resistance of the sample the more curved the lines. Figures at bottom represent seconds of time required for kymograph pen to trace the curve, using a uniform mercury flow.

the gluten being dipped under 0.1% sodium phosphate solution (pH 6.8) between tests to avoid drying out. It was found that the final tests on each sample indicated a slight stiffening of the gluten due apparently to manipulation.

A typical record of the resistance test is shown in Figure 10. The values shown are the times taken for the kymograph pen to travel from the start of application of load to the 0.175-inch line, a distance of 0.225 inch. This point was chosen because it occurs after the line has indicated the type of curve and before it has reached the region of ex-

cessive flattening out of the gluten ball where its power of resistance to the deforming force is largely overcome.

The average value for the five determinations was then calculated and used as a single-figure value for a measure of the resistance. Two sets of curves constructed from such average values are shown in Figure 11. It will be noticed that the curves at the left are straighter, *i.e.* rise more rapidly, than the curves at the right, indicating less resistance of the gluten due to the effect of papain as compared with the increased resistance contributed by potassium bromate presumably through inhibitory action upon flour and yeast proteases. Little difference is to

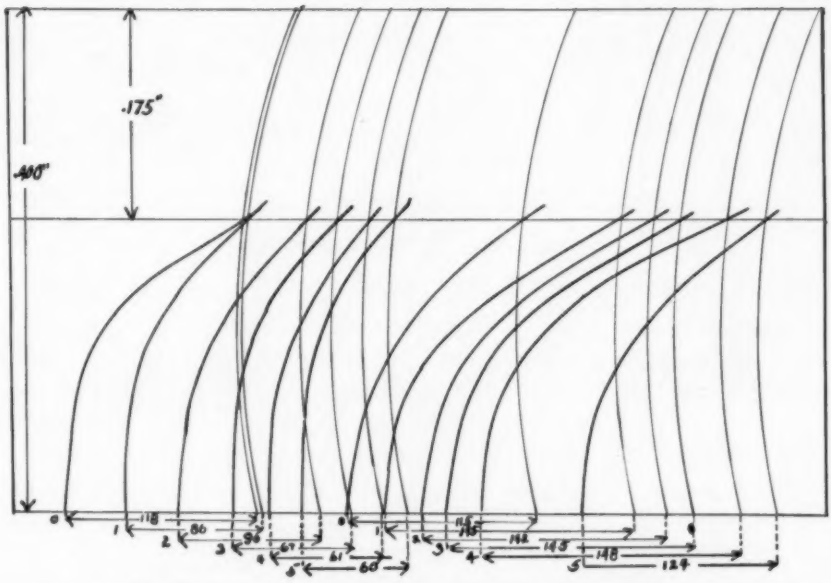

Fig. 11. Resistance curves showing influence of ingredients and fermentation time upon shape of curve. Curves at left represent results obtained with the standard basic formula plus 0.004% papain. Curves at right were obtained with the standard basic formula plus 0.001% $KBrO_3$. Number of the curve refers to hours of fermentation.

be noted between the curves representing different hours of fermentation in the first set of curves, but when $KBrO_3$ was added to the basic formula the lines tended to become more curved with length of fermentation period until the fourth hour, indicating that this flour improver definitely stiffens the dough as fermentation progresses. This evidence is in line with baking observations.

Figure 12 represents the changes in resistance with length of fermentation period for the six doughs. Comparing first the two formulas without papain, the stiffening effect of bromate after the first hour of

fermentation becomes apparent. Why the dough without bromate should be initially stiffer than the bromated dough is not clear. Next, in comparing the effects of the two papain increments upon the standard basic formula, it is apparent that the enzyme decreases the resistance of the gluten from the moment the dough is mixed, and that this effect increases with length of fermentation period. This again is in agree-

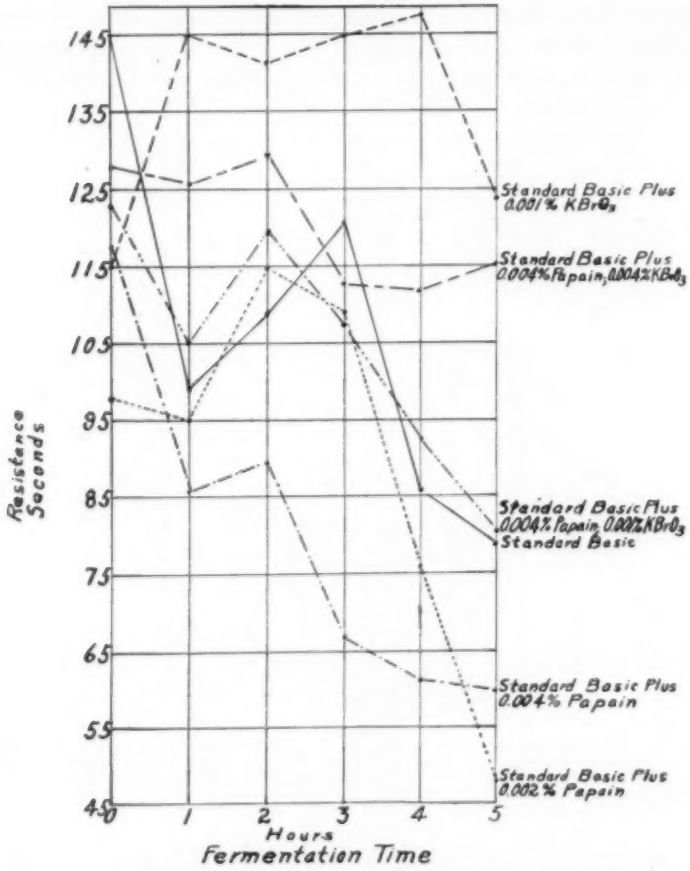

Fig. 12. Change of resistance in seconds of glutens prepared from doughs fermented varying lengths of time.

ment with baking observations which offer evidence of progressive softening of the dough as fermentation progresses in the presence of papain. When bromate is added to the papain-treated doughs a decided stiffening of the gluten is noticeable, especially as fermentation progresses. The data graphically presented in this figure show the same trends in regard to papain and bromate effects as were evident in the viscosity deter-

minations. It is therefore apparent that the physical and biochemical properties of the gluten are affected in comparable manner by either papain or bromate additions.

Correlation coefficients presented in Table II show that a relatively high relationship existed between viscosity and the quantity of protein removed from colloidal solution by $MgSO_4$. The influence of varying fermentation time no doubt lowered this correlation as compared with glutens washed from flour-water doughs.

TABLE II

CORRELATION COEFFICIENTS COMPUTED FROM THE DATA

Variables correlated		Correlation coefficient	
X	*Y*	*r xy*	*P*
Viscosity (centipoises $\times 10^2$)	Protein fractionated (mg.)	+.6960	<.0001
Viscosity (centipoises $\times 10^2$)	Resistance of gluten (seconds)	+.5547	.0006
Resistance of gluten (seconds)	Protein fractionated (mg.)	+.3043	.0730

Summary and Conclusions

A series of doughs was prepared from a commercial spring wheat flour using six formulas. These formulas included the standard basic (with 5% sucrose), standard basic plus 0.001% $KBrO_3$, standard basic plus 0.002% and 0.004% papain, and the latter two formulas plus 0.001% and 0.004% $KBrO_3$, respectively.

Three series of glutens were washed by standardized methods from the doughs following fermentation periods ranging from 0 to 6 hours for each formula. Comparative rates of dispersion of the glutens in 10% sodium salicylate were determined on one set, as indicated by changes in viscosity with time. The results showed that glutens prepared from doughs without added papain tended to disintegrate at a lower rate after three hours of fermentation than before, while bromate greatly reduced the rate after two hours of fermentation. The addition of papain without bromate accelerated the dispersion rate and obliterated any effect of fermentation time upon this variable. When $KBrO_3$ was superimposed upon papain, however, fermentation behavior reflected the inhibitory action of the oxidizer upon the enzyme.

In a second series, glutens were washed from the doughs, then dispersed, and the protein concentration adjusted to 2500 mg. per 100 cc. of dispersion. The viscosities were determined, and the resulting data showed the effect of papain in reducing this property. There was a marked tendency for the viscosity to increase to a maximum after two

to three hours of fermentation of the dough. Unbromated doughs showed some evidence of an initial decrease in viscosity during the first hour of fermentation. In the presence of bromate this behavior was less apparent. Viscosity decreased after three hours of dough fermentation in every instance. The results obtained by fractionating the protein from the dispersions by suitable additions of $MgSO_4$ revealed a somewhat similar picture when due allowance was made for the variability inherent in the biologic material and methods used. Doughs treated with papain showed initial decreases in the quantity of protein removed, corresponding to the viscosity trends. Why such a marked trend should be established only in the unfermented doughs is not clear. Bromate appeared to increase the quantity of protein fractionated in the presence of papain at the beginning and end of the fermentation, but whether it had any effect upon the results obtained at the second or third hour is very doubtful. The 0.004% $KBrO_3$ treatment did show a high point at four hours of fermentation, but whether this points to the bromate having a maximum effect at this time cannot be definitely stated.

A third series of glutens was likewise prepared and resistance tests run on glutens molded into small balls using a modification of the technique developed by Binnington, Johannson and Geddes (1939) for measuring the " tenderness " of cooked macaroni.

The data obtained, when plotted against fermentation time, showed a decrease in resistance followed by an increase which reached a maximum at the second or third hour in every dough except one, the standard basic plus 0.001% $KBrO_3$. The gluten from the 0.004% papain treated dough increased little in resistance between the first and second hours, and then decreased sharply. The stiffening effect of $KBrO_3$ upon these glutens is quite marked, and tends to increase with the fermentation period. The results obtained with this test are in general agreement with the other determinations employed and illustrate the inhibitive effect of $KBrO_3$ upon proteases during dough fermentation. It is not clear from the data whether there is a direct effect of the oxidizing agent upon the gluten properties. The action of papain in increasing the rate of gluten dispersion and in decreasing the viscosity of gluten dispersions, as well as the quantity of gluten fractionated from the dispersions, is clearly evident, as well as its effect in decreasing the resitance or stiffness of the gluten as fermentation proceeds.

Correlation coefficients showed a significant relationship between viscosity and protein fractionated from the dispersions.

Literature Cited

Bailey, C. H., and Johnson, A. H.
1924 Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. Cereal Chem. **1**: 293–304.

Binnington, D. S., Johannson, H., and Geddes, W. F.
1939 Quantitative methods for evaluating the quality of macaroni products. Cereal Chem. **16**: 149–167.

Bohn, L. J., and Bailey, C. H.
1936 Elasticity of wheat flour dough. Cereal Chem. **13**: 389–409.
1937 Effect of fermentation, certain dough ingredients, and proteases upon the physical properties of flour doughs. Cereal Chem. **14**: 335–348.

Brownlee, W. E., and Bailey, C. H.
1930 Proteolysis in bread doughs. Cereal Chem. **7**: 487–517.

Flohil, J. T.
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. Cereal Chem. **13**: 675–689.

Harris, R. H.
1938 A study of the effect of proteoclastic enzymes upon wheat gluten solubility in sodium salicylate solutions. Cereal Chem. **15**: 690–707.

—— and Johnson, John, Jr.
1940 A study of the effects of proteolytic enzymes and $KBrO_3$ upon the viscosity and allied properties of dispersions of hard red spring wheat gluten. Cereal Chem. **17**: 203–222.

—— and Knowles, Darline
1940 Quality studies on North Dakota durum wheat. Cereal Chem. **17**: 480–490.

Henderson, L. J., Fenn, W. O., and Cohn, E. J.
1919 Influence of electrolytes upon viscosity of dough. J. Gen. Physiol. **1**: 387–397.

Jørgensen, Holger
1935 Über die Natur der Bromatwirkung. Mühlenlab **5**: 113–126.
1936 On the existence of powerful but latent proteolytic enzymes in wheat flours. Cereal Chem. **13**: 346–355.

Olsen, A. G., and Bailey, C. H.
1925 A study of the proteases of bread yeast. Cereal Chem. **2**: 68–86.

Read, J. W., and Haas, L. W.
1937 Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. Cereal Chem. **14**: 752–766.
1938 V. Further studies concerning potassium bromate and enzyme activity. Cereal Chem. **15**: 59–68.
1939 VI. Further studies relating to the activation and inhibition of flour proteinase. Cereal Chem. **16**: 60–70.

Sharp, P. F., and Elmer, R.
1924 Wheat and flour studies. I. Proteolytic enzymes of flour. I. Autodigestion of flour milled from frozen and non-frozen wheat harvested at various stages of maturity. Cereal Chem. **1**: 83–106.

—— and Gortner, R. A.
1924 The physico-chemical properties of strong and weak flours. VIII. Effect of yeast fermentation on imbibitional properties of glutenin. Cereal Chem. **1**: 29–37.

—— and Schreiner, O. M.
1926 Wheat and flour studies. VI. Effect of yeast fermentation on the proteins of flour. Cereal Chem. **3**: 90–101.

Smith, E. E.
1925 Viscosity and baking quality. Cereal Chem. **2**: 177–190.

THE INFLUENCE OF THE INITIAL pH OF RYE MASHES ON THE FERMENTATION EFFICIENCY [1]

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The efficiency of rye fermentations (about 83%–86%) had never equaled the efficiency of the corn spirits fermentation (90%–93%) in our plants, and to the best of our knowledge this is the common experience of other plants. The difference in efficiency has been explained in many ways. One possibility is that rye contains a higher percentage of pentosans and other materials which react as starch in an acid-hydrolyzed sample; another, that rye mashes which are not cooked under pressure as is corn, have a greater initial contamination than corn spirits mashes; and there are other possible explanations. Both of the above explanations are plausible. Rye as received has been found to contain a higher percentage of pentosans than corn, and since the maximum temperature of mashing rye is 145° F. (in our procedure), many contaminants capable of development in the fermenter are left in the mash. Most of the other reasons advanced in explanation of the low efficiencies with rye fermentations now appear to have little or no foundation.

Calculations as to the validity of the first explanation for lower efficiency revealed that it could not account for the entire difference. Attention was directed to the fact that the initial pH of a rye mash is about 5.4–6.0, while corn spirits fermentations have a pH of 4.9–5.0 because of the addition of stillage or acid. It was considered probable that this high initial pH of a rye mash permitted large numbers of the contaminants which had survived mashing to grow and produce acid. This acid production would not only be at the expense of the carbohydrate, but moreover might result in the destruction of free amylase activity by lowering the pH of the mash below 4.0 and thus prevent the complete conversion of residual dextrines to maltose.

The results of laboratory tests made by Mr. J. S. Hudson [2] of our department indicated that the yield of alcohol and efficiency of the fermentation were improved by adjusting the initial pH of the mash to 4.8–5.0 with sulphuric acid. This was indirectly corroborated by Kendall (1938) who has recently reported improved alcohol yields during the fermentation of rye mashes by the addition of a small amount of lactic acid, and he assumed that the effect was the result of reduced contami-

[1] This paper was presented at the annual meeting of the Society of American Bacteriologists, New Haven, Conn., December 28, 1939.

[2] Private communication.

nation. However, it was his belief that the cost of the lactic acid would not be recovered in the additional yield of alcohol. Nevertheless, before attempting to introduce pH adjustment into an operating plant it was necessary to conduct experimental fermentations on an intermediate scale. The results of these experiments are reported here.

Experimental

A series of experiments was conducted at the Lawrenceburg, Indiana, plant and the mashes used in the experiments were portions of the regular mashes prepared at that plant. The rye mashing procedure used is as follows: The water is drawn into the pony mash tank at a temperature of 110° F., the malt is added while the water is agitated, and the slurry is then added to the cooker which contains water at a temperature of 100° F.; after all the malt is added, finely ground rye is introduced slowly while the mash is constantly agitated. The mash is mixed for 10 minutes at this temperature. The temperature is then raised to 130° F. in 30 minutes and held for 45 minutes, thus allowing some proteolysis and some conversion of starch, which results in a thinner mash. The temperature is then raised to 145° F. in 15 minutes and held for one hour; at this time the mash is pumped through the coolers and into the fermenters. Four cooks and the yeast mash fill the fermenter. The final volume and setting temperature are adjusted with stillage and water. The yeast is usually added to the fermenter with the first cook. This addition is made about 4 to 6 hours before the fermenter is completely filled with the other three cooks and the stillage or water.

For the experimental work, as soon as all of the rye mash was in the fermenter and usually before any stillage or water was added, 50 gallons of mash was removed from the fermenter, thoroughly mixed, and split into two 25-gallon portions which were placed in 55-gallon drums that had just been steamed for one hour. The drums were then placed next to the parent plant fermenter. By the addition of 6 normal sulphuric acid the pH of one of the two small fermenters was lowered to a pH between 4.8 and 5.0. To the second fermenter an equal volume of distilled water was added to correct for the dilution of the other mash and this served as the unadjusted control. Both fermenters were then covered with the lids of the drums. In this manner such variables as differences in grains, mashing conditions and concentrations, yeast used for inoculum, and the incubation temperature were eliminated. The pH, Balling, and titratable acidities were determined, and every 24 hours the same determinations were repeated. Occasionally it was necessary to omit the usual three-hour pH and temperature determinations. The fermenters were sampled for final data at the time

the parent plant fermenter was pumped to the beer well for distillation. This was after approximately 72 hours. The final data consisted of duplicate alcohol determinations, pH, Balling, titratable acidity, and residual sugar determinations. The alcohol content was determined by distillation of a known volume of mash, followed by the analysis of the distillate with a Zeiss refractometer. The residual sugars were determined on samples of supernatant liquid from centrifuged samples. Each sample was acid hydrolyzed and the sugar content determined by the method of Stiles, Peterson, and Fred (1926).

Table I lists the results of eight experiments. The variations in the final alcohol content among experiments are due to variations in the

TABLE I

A SUMMARY OF THE PERTINENT DATA ON 25-GALLON EXPERIMENTAL RYE FERMENTATIONS WITH THE pH ADJUSTED AND ON THE CONTROL FERMENTATIONS

Experiment	Fermenter [1]	Initial pH	Final data			
			pH	Titratable acidity	Residual sugar	Alcohol content
				% acetic	*g./100 cc.*	*% by vol.*
1	a	5.50	3.75	0.68	0.96	5.17
	b	5.11	3.76	0.63	0.96	5.30
2	a	5.38	3.75	0.66	1.05	5.37
	b	4.85	3.75	0.62	1.00	5.53
3	a	5.45	3.72	0.71	1.14	5.83
	b	4.88	3.73	0.67	1.06	5.99
4	a	5.45	3.72	0.68	1.10	5.24
	b	4.88	3.78	0.64	1.04	5.37
5	a	5.58	3.70	0.69	1.31	5.20
	b	4.70	3.82	0.59	1.02	5.63
6	a	5.50	3.58	0.87	1.39	5.41
	b	5.10	3.73	0.74	0.92	5.52
7	a	5.37	3.65	0.87	1.15	5.12
	b	5.00	3.81	0.68	1.02	5.52
8	a	5.72	3.68	0.82	0.96	5.43
	b	4.98	3.75	0.73	0.84	5.63

[1] Fermenter *a* served as the control (pH unadjusted), the pH of fermenter *b* was adjusted to 4.8–5.0 in all cases at the beginning of the experiment.

concentrations of the mash used. This was the result of sampling the plant fermenters after varying amounts of stillage or water had been added to them. Figure 1 is a representative graph showing the pH curves for the experimental fermenters and for the plant fermenter, re-

spectively. Toward the end of the experiment it was impossible to obtain data every three hours; hence there are fewer points on the graph for the final stages of the fermentation. As may be noted from Table I the alcohol content was higher in every case in those fermentations with the pH adjusted to 4.8–5.0. This average increase is 0.22% by volume of alcohol. Thus the efficiency of the fermentation was raised by adjustment of the initial pH to 4.8–5.0. The variations between sets of fermentations are due to the fact that it was impossible to sample the plant fermenters when the same dilution of the mash with stillage had occurred each time and thus the initial sugar content varies

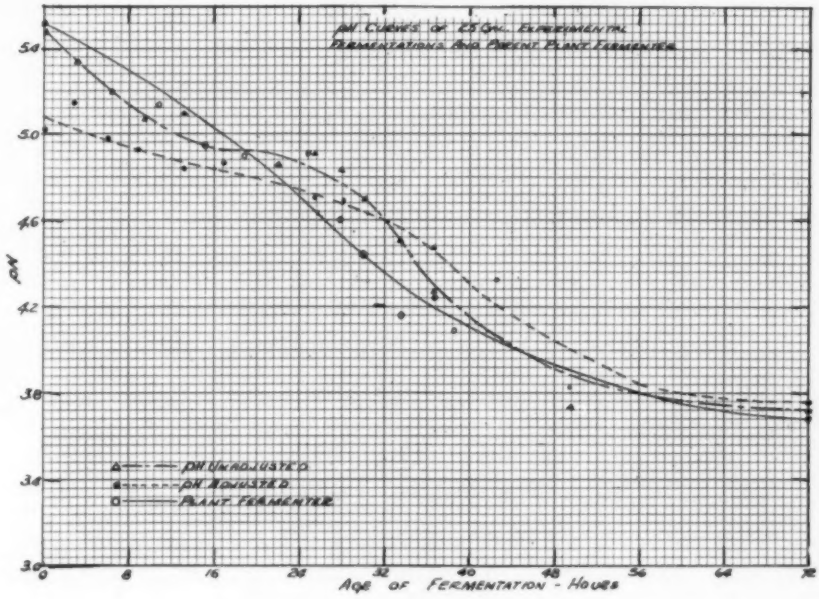

Fig. 1.

from experiment to experiment. Table I also shows that with the exception of Experiment 1, less residual sugar is present in those fermentations in which the initial pH was adjusted.

One of the best indications of the degree or extent of contamination is the titratable acidity in the fermentation; a high acidity indicates greater contamination. As may be seen, the final titratable acidity is higher in all experiments when the initial pH of the fermentation is unadjusted; thus it would appear that initial pH adjustment prevents contamination and acid production.

As a result of the evidence presented in Table I it was decided to adopt pH adjustment of rye fermentation mashes in plant practices.

This was done, and by the addition of from 5 to 8 gallons of 66° Be. sulphuric acid to 40,000 gallons of mash the initial pH was lowered to 4.8–5.0. The results of eight plant fermentations, using this procedure, are presented in Table II. The efficiency (91.5%) was calculated from the starch analysis of the grain and the actual yield of alcohol from plant distillation. This efficiency with rye fermentation is the highest, by 5%–6%, ever obtained in our plants or in others, to the best of our knowledge, and has been successfully maintained since the introduction of initial pH adjustment.

TABLE II

EIGHT RYE MASH PLANT FERMENTATIONS IN WHICH THE INITIAL pH WAS ADJUSTED
Mash Bill—80% Rye, 2% Barley Malt and 18% Rye Malt

Experiment	Initial pH	Alcohol yield (proof gallons/bushel of grain) Analytical	Actual
1	4.96 5.03 distilled 4.83 together 5.04	4.76	4.68
2	4.87 distilled 5.04 together	4.69	4.71
3	4.97 distilled 4.93 together	4.62	4.64

Average fermentation efficiency = 91.5% of theoretical calculated from the actual plant yield.

Discussion of the Data

As previously stated it was believed that the degree of bacterial contamination with resultant acid production and loss of free amylase activity was largely responsible for the low alcohol yields in rye fermentation. The data presented in this paper support this theory. In Figure 1 it will be seen that the pH curves for the unadjusted fermentation and the plant fermentation are very nearly identical. The pH remains above 5.0 for the first 12 to 16 hours during which time more contaminants may develop than in the fermentation with the pH adjusted. After 20 to 24 hours the pH for the unadjusted fermentation falls below the level of the adjusted fermentation and then falls below 4.0 at the end of 40 hours, which is about 4 hours earlier than for the fermentation with the initial pH adjusted. Below a pH of 4.0 free amylase activity disappears. Occasionally the pH of the adjusted fermentation will fall below 4.0 at about the same time as the unadjusted fermentation; this is, of course, affected to some extent by other factors, *i.e.* initial contamination, mashing conditions, etc. None the less, a fermentation with the

initial pH unadjusted is subjected to more opportunity for the growth of acid-producing contaminants (which apparently develops) than one in which the initial pH is lowered to 4.8–5.0, and the unadjusted mash is ordinarily in a pH range favorable to amylase activity for a comparatively shorter period of time.

The foregoing postulations are well supported by the data in Table I. The lower final titratable acidity values in the eight fermentations indicate less contamination with acid-producing bacteria and the lower residual sugar content in the eight groups of fermentations shows that amylase activity has been greater, with the result that more dextrine was converted to maltose which was subsequently fermented.

Summary

It has been shown that by adjusting the initial pH of rye mashes to 4.8–5.0 through the addition of sulphuric acid to the mash the efficiency of the fermentation was increased by approximately 4% in a series of test fermentations. This increase in efficiency appears to be due to a reduction in the growth of contaminants, with comparatively less loss of carbohydrate, with less acid production, and presumably with a greater protection to the amylase activity.

When this technique was placed in plant practice the efficiency of plant-scale rye fermentations was raised from 83%–86% to 90%–92%.

Acknowledgment

The authors gratefully acknowledge the assistance of Mr. J. S. Hudson, Mr. R. E. Scalf, Mr. Frank Gallagher and Mr. G. A. Ratti of the Seagram Research Department in conducting this series of experiments.

Literature Cited

Kendall, A. R.
1938 The fermentation of rye. Proc. S. D. Acad. Sci. **18**: 26–36.
Stiles, H. R., Peterson, W. H., and Fred, E. B.
1926 A rapid method for the determination of sugar in bacterial cultures. J. Bact. **12**: 428–435.

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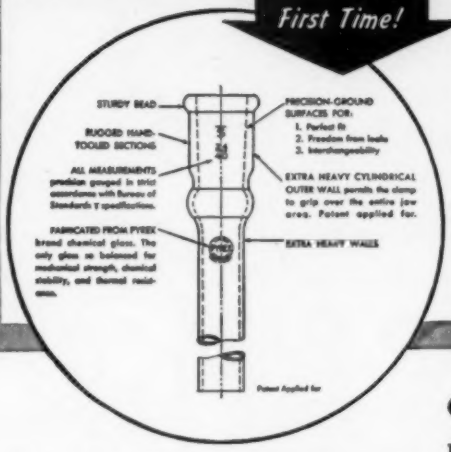
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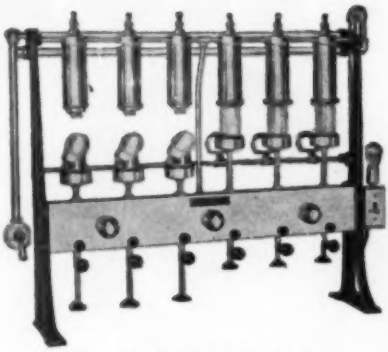

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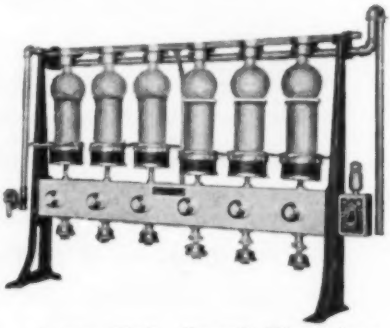

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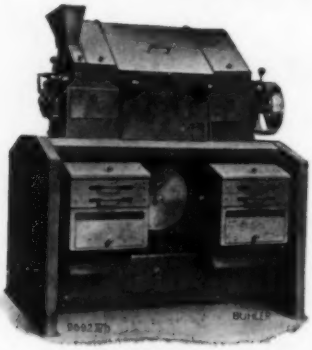

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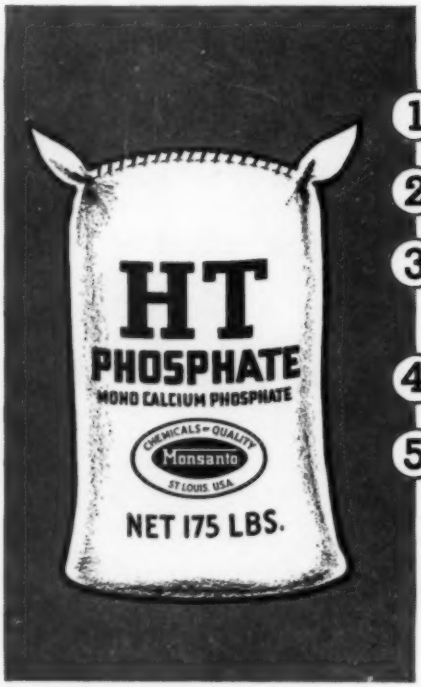

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